THE INHERITANCE OF SHANK COLOR IN CHICKENS

C. W. KNOX
Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.

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Several publications in recent years have given conflicting reports upon the inheritance of shank color. Yellow shank color has been reported to be inherited in a simple Mendelian fashion, the sex-linked gene for yellow shank color (Y) being dominant to its allele (y), the gene for blue shank color. In contrast to this, the gene for yellow shank color has been reported to be autosomal, the recessive gene (w) designating yellow shanks and the dominant gene (W) representing white shanks. Obviously, these two assumptions are in disagreement. Still another hypothesis is that yellow shanks carry a dominant sex-linked inhibiting gene for dark shank color (D), while dark shanks lack this inhibitor (d). In other words, the following situation prevails:

Dominant gene	Allele	Chromosome
W = white shanks	w = yellow shanks	autosomal
Y = yellow shanks	y = blue shanks	sex-linked
D = inhibitor of dark shank color	d = dark shanks non-inhibitor	sex-linked

WHITE AND YELLOW SKIN COLOR

PUNNETT (1923), in writing of white and yellow skin color, has stated that yellow and white appear to behave as a simple pair of characters, the latter being dominant to the former. A few years later, DUNN (1925) reported a similar situation in regard to the inheritance of yellow and white shank color. The factor for white shanks in DUNN'S (1925) report is assigned the symbol W, and he states that this gene is practically dominant to the gene for yellow designated as w. Two years later DUNN and JULL (1927) and LAMBERT and KNOX (1927) verified this theory. In addition to this, LAMBERT and KNOX (1927) have stated that this one autosomal gene affected the color of the skin, beak and shanks.

In contrast to this, WARREN (1928) has stated that the gene which determines shank color is very definite in its expression but is probably masked by certain plumage colors. The contrasted characters were yellow and blue shanks, which he designated as Y for yellow and y for blue shanks, and also stated that these factors were sex-linked.

From previous investigations it is apparently agreed that there are two kinds of pigments. HANAU (1881), JEFFRIES (1883), and GADOW (1891) state that the various colors of the shank are due to the presence or absence

of two kinds of pigment, orange-yellow and brownish-black. They agree that the orange-yellow is a lipochrome pigment which, in concentrated form, gives an orange-yellow color and in a more diluted form a light-yellow, even to an almost whitish color. The brownish-black pigment is in reality a melanic pigment and is responsible for the black, blue, and green shades.

The histological work of BARROWS (1914) verified this and showed, in addition, that the colors are varied according to the amount and kind of pigment and whether or not the pigment is deposited in the dermis or epidermis of the skin or both. It is evident from these investigations, therefore, that two kinds of pigments are concerned in shank color.

It is universally known that all breeds and varieties of chickens have yellow or white skin color, that is, the presence or absence of lipochrome pigment. Sometimes the melanic pigment in the shanks is so dark and so completely covers the shanks that it is difficult to ascertain the presence or absence of lipochrome pigment. In such cases, this can be done readily by observing the skin color of the bottom of the feet. For example, Jersey Black Giants have very black shanks but the bottom of the feet have some lipochrome pigment appearing through the black, whereas the Black Langshan has just as black shanks as the Jersey Black Giant but the bottoms of the feet have some white appearing through the black, showing the lack of lipochrome pigment.

In a study of the inheritance of lipochrome color, a backcross was made of an F_1 male (Buff Orpington×Barred Plymouth Rock) with Rhode Island Red females. The genotypes involved were ♂ *Ww*× ♀ ♀ *ww*. Half of the progeny would be expected to have a lack of lipochrome pigment, as indicated by the white shanks, and the other half would be expected to have yellow shanks indicating the presence of lipochrome pigment. The results obtained were 516 progeny without lipochrome pigment in the shanks and 448 with lipochrome. This was a deviation of 34 from the theoretical number expected, 482. Most of this deviation was due to the fact that the chicks were raised in batteries, which would tend to bleach the yellow shanks so that in some cases these would be classed as lacking lipochrome. It was further complicated by the range in shank color in both groups. The range in color was from an ebony black to a clear white or yellow.

It is evident from the previous investigations mentioned and this one that yellow pigment in the shanks is inherited in a simple monohybrid manner and is autosomal, being recessive to its allele, *WW*, the lack of lipochrome pigment in the shanks. Hence all breeds and varieties either have yellow pigment, *ww* (presence of lipochrome), or lack yellow pigment, *WW* (absence of lipochrome), in the shanks.

The only exception to this was the result reported by WARREN (1928), who stated that yellow (*YY*) was dominant to blue (*yy*) shanks. He probably arrived at this result due to the complication of blue shank color. If he had considered yellow and blue shank color in the same manner as DUNN and JULL (1927) he might have been in accord with the statement made in the preceding paragraph. Therefore, it is the writer's belief that the work of WARREN (1928) would more correctly fit in a study of the inheritance of dark rather than yellow shank color.

DARK AND LIGHT SHANK COLOR

Several conflicting theories in respect to the mode of inheritance of dark shank color have been advanced. However, as previously stated, dark shanks are caused by melanic pigment. The presence or absence of various amounts of melanic pigment and whether or not it was deposited in the dermis or epidermis or both, as well as the presence or absence of lipochrome pigment tend to make the whole problem complicated. The different combinations of these pigments or their absence are responsible for the various shades of shank color from white or yellow, blue or green, to the deepest color, the ebony black.

The best and most probable explanation of shank color is reported by DUNN and JULL (1927). In crosses involving the White Silkie, they reported that the genes involved in shank color were essentially as follows:

	MALES	FEMALES
White, pinkish white, pearl and light blue shanks, (may show spots of dermal pigment especially in the females and *Dd* males)	*WWDD* *WwDD* *WWDd* *WwDd*	*WWD–* *WwD–*
Orange yellow, light yellow, creamy shade (may show spots of dermal pigment in females and in *Dd* males)	*wwDD* *wwDd*	*wwD–*
Black, slate, blue and gray shanks	*WWdd* *Wwdd*	*WWd–* *Wwd–*
Black, dark green, light green or willow shanks	*wwdd*	*wwd–*

This classification is based upon two sets of factors, one autosomal for white and yellow color, and the other sex-linked and controlling dark shank color. Whether the genotypes given above cover all cases remains to be determined. PUNNETT (1923) suggested that there are indications that the presence of dermal pigment in certain instances is dominant to its absence. JULL (1932) states that "since it has been suggested that highly developed epidermal pigment is associated with black or dark brown plumage, it is possible that the gene *E*, for the extension of melanic pig-

ment throughout the plumage, may also affect the extension of melanic pigment to the shanks."

It has been shown that there are several ways in which the melanic pigment might be inherited. First, as stated by WARREN (1928) yellow is sex-linked and dominant to blue; second, the suggestion of PUNNETT (1923) that the presence of dermal pigment is dominant to its absence; third, as JULL (1932) suggests, the gene *E* for the extension of black in the plumage may be of a general nature and may affect the shank color; fourth, as classified by DUNN and JULL (1927).

RESULTS

With these assumptions in mind, data were compiled at the U. S. ANIMAL HUSBANDRY EXPERIMENT STATION, Beltsville, Maryland, in an attempt to clarify the inheritance of dark shank color. The problem appeared to be one involving the presence or absence of melanic pigment and the birds were so classified, omitting the yellow and white classification and making no attempt to differentiate dermal from epidermal melanic pigment. In this way complications were avoided that arose from the blue, green, and black shank colors.

THE EFFECT OF THE GENES *EE* AND *BB* UPON MELANIC SHANK COLOR

It has been observed in publications by BATESON and PUNNETT (1911), DUNN (1925), DUNN and JULL (1927), and WARREN (1928), that in crosses where the results secured were interpreted on a sex-linked basis such crosses involved a sex-linked plumage factor, usually that for barring. Therefore, in this experiment the first cross made was between Jersey Black Giants, which had black shanks, and Rhode Island Reds, which lacked black pigment in the shanks. Both breeds had the genes *ww* for yellow shank (skin) color so that this factor was eliminated in this cross. The plumage genes involved were mainly *EE* for black plumage and *ee* for the non-extension of black plumage, plumage genes that are not sex-linked.

The plumage of all of the 118 F_1 (*Ee*) progeny from reciprocal matings was black or predominantly black, showing no sex-linkage, and all showed varying amounts of melanic pigment in the shanks. There was more red in the plumage color of the F_1 males than in the plumage of the F_1 females, and about 40 percent of the plumage of 3 of the F_1 males was red; one of these males had yellow shanks with a tinge of melanic pigment, the other two males had shanks that were a dusky yellow. One of the F_1 males with black plumage had dark green shanks. All of the remainder of the F_1 progeny had predominantly black plumage and dark shanks. Thus it would appear that the melanic pigment in the shanks of this cross was dominant to yellow shanks, as suggested by PUNNETT (1923), and in

contrast to the results reported by WARREN (1928). This also agrees with the suggestion of JULL (1932) that the plumage gene *E* has some effect on the melanic color in the shanks. As a matter of fact, this cross shows that the gene *E* for the extension of melanic pigment is of a general nature and effects the deposition of melanic pigment in the shanks in about the same manner that it does the plumage color.

As further proof of this statement, three Jersey Black Giant females mated to a Rhode Island Red male proved to be heterozygous blacks, *Ee*, and produced 18 predominantly black offspring, (*Ee*), and 10 red (*ee*) offspring. Of the 18 black offspring, 17 had black shanks and one male with a considerable amount of red plumage had dusky yellow shanks. All of the red progeny, males and females, had yellow shanks devoid of melanic pigment. If there were separate genes for the deposition of melanic shank color in this cross, the red progeny, *ee*, would have had black shanks, the same as their black sisters. Of course, this does not obviate the possibility of a close linkage between black plumage color and dark shank color. Such a linkage would fix the dark shank color on the autosome carrying black rather than on the sex chromosome, as previously reported. However, it seems more likely and logical that the genes, *EE*, have a general effect upon the deposition of melanin, which would include the plumage, shanks, beak, and possibly dark eye color.

From a backcross mating of a Rhode Island Red male (*ee*) with F_1 (*Ee*) females (Jersey Black Giants × Rhode Island Reds), 260 red, 11 columbian, and 225 black progeny were produced. The 260 with red plumage color had yellow shanks, lacking any sign of melanic pigment in the shanks. The same was true of the 11 columbian-patterned progeny. There was considerable variation in the plumage and shank color of the 225 predominantly black progeny, most of which had dark shank color. It would appear, therefore, that while the gene *E* effects the deposition of the melanic pigment in the plumage and the shanks, there is considerable variation in the shank and plumage color in the heterozygous black (*Ee*) progeny. This variation is due, no doubt, to the effect of modifying factors and possibly different hormone activity, especially as between sexes. It was noted again that some of the F_1 males usually carried less black pigment in the plumage and less melanic pigment in the shanks than the F_1 females.

A similar condition was noted in 153 F_2 black and 49 F_2 red progeny from a Jersey Black Giant × Rhode Island Red parental cross. All of the birds with red plumage color had yellow shanks, whereas the progeny with black plumage had dark shank color. The amount of black pigment in the shank color of these birds closely approximated the amount of black observed in the plumage color.

In another backcross of Rhode Island Red females with an F_1 male (Buff Orpington male×Barred Plymouth Rock females), it was found that the barring factor affected not only the black plumage genes, *Ee*, restricting the black to alternate bars of black and white, but also practically eliminated the evidence of melanic pigment in the shanks. All of the progeny from this cross were classified with respect to plumage color as black and white barred, gold and white barred, black, gold, and columbian. Each group had the shank color classified as dark, medium and clear. Only black and almost black shanks were classified as dark, whereas the medium dark colored shanks included blue, green, dusky yellow, dusky white, light green and light blue, and the clear shanks included only the white and yellow shanks that lacked melanic pigment. The observed results are given in table 1.

TABLE 1

Plumage and shank color of backcross offspring, F_1 male (Buff Orpington male×Barred Plymouth Rock females)×Rhode Island Red females.

PLUMAGE COLOR OF OFFSPRING	SHANK COLOR								
	MALES			FEMALES			TOTALS		
	DARK	MEDIUM	CLEAR	DARK	MEDIUM	CLEAR	DARK	MEDIUM	CLEAR
Black and white barred *S-B-Ee* and *s-B-Ee*	2	9	105	1	15	69	3	24	174
Gold and white barred *s-B-ee*	0	0	71	0	0	49	0	0	120
Black *S-b-Ee* and *s-b-Ee*	44	29	23	48	28	15	92	57	38
Gold *s-b-ee*	0	3	95	0	4	59	0	7	161
Columbian (Silver) *S-B-ee* and *S-b-ee*	0	0	134	0	0	88	0	0	222

The data in table 1 show that only 7 of the 510 *ee* progeny from this backcross had medium dark shanks, the remaining 503 showed no melanic pigment. The 7 *ee* progeny were golds and showed a considerable amount of black, which might account for the medium dark shanks. However, the plumage of these birds were predominantly red. The data show that the 120 *ssBbee* and *s-B-ee* progeny (*B*– barring; *S*– silver; *b*– non-barred; *s*– non-silver; *E*– extension of black plumage; *e*– non-extension) had no melanic pigment deposited in the shanks. However, out of 210 black and white barred progeny 3 were classified with dark shanks and 24 with medium dark shanks. The 201 progeny were obviously of the genotype *BbSsEe*, *S-B-Ee*, *ssBbEe*, and *s-B-Ee*. The 187 predominantly black progeny, on the other hand, were mostly dark-shanked birds, 149 having dark or medium dark shanks. Only 38 of the black progeny had no melanin present in the shanks. These 38 individuals may have non-melanic shank color because they are heterozygous blacks and approximately half of them

would be heterozygous for such modifying factors as silver, *S-* or *Ss*, and the other half golds, *s-* or *ss*. These data demonstrate that the barring factor, *B*, affects the genes *EE* for the extension of black in the plumage and also has the effect of restricting the deposition of melanic pigment in the shanks.

The data in table 2 give the results obtained from an F_1 male (Buff Orpington male×Barred Plymouth Rock females) crossed with White Plymouth Rock females, and these data verify the statement made in the preceding paragraph. Of the 100 individuals from this mating 22 were non-barred, black birds (*Ee*) that had dark or medium dark shanks, whereas the 47 non-barred red and columbian progeny (*ee*) had clear white or yellow shanks. The 31 black and white barred group had 8 with medium dark shanks and 23 with clear white or yellow shanks. These results again show the effect of the gene *E* upon shank color and the restricting effect of the barring gene *B*.

TABLE 2

Plumage and shank color of backcross offspring, F_1 male (Buff Orpington male×Barred Plymouth Rock females)×White Plymouth Rock females.

PLUMAGE COLOR OF OFFSPRING	SHANK COLOR								
	MALES			FEMALES			TOTALS		
	DARK	MEDIUM	CLEAR	DARK	MEDIUM	CLEAR	DARK	MEDIUM	CLEAR
Black and White barred	0	4	14	0	4	9	0	8	23
Black	1	2	0	9	10	0	10	12	0
Gold	0	0	11	0	0	8	0	0	19
Columbian (Silver)	0	0	17	0	0	11	0	0	28

In another cross, Rhode Island Red males with Barred Plymouth Rock females, the F_1 male progeny were barred, all having yellow shanks, and the F_1 females were predominantly black with dark shanks. If the barring gene *B* acted as a restrictor of melanin in the plumage and in the shanks, as previously mentioned crosses show, this apparent sex-linkage of dark shank color would be expected. The cause, however, could not be attributed to sex-linkage of a dark shank color gene without considering the effect of the sex-linked barring gene, which has a restricting effect on the black color in the shanks as well as the black plumage color. Even in this cross, however, the data on 145 black female progeny show that the percentage of black plumage and the percentage of melanic pigment in the shanks are significantly correlated, being +0.72. It was also noted that the standard deviation from the mean of the plumage color was 22.1 percent and the standard deviation for shank color was 22.2 percent. In other words, there was as much variation in the plumage color of these F_1

females which were of the genotype *b-EeCC* as there was in the shank color. It has been found that in most plumage color crosses, the F_1 progeny vary considerably in plumage color and it is probable that the variation of plumage and shank color found in the F_1 black females merely expresses an expected normal variation.

It is evident from the results mentioned, that WARREN (1928), who used crosses involving barring, *BB*, (Barred Plymouth Rocks and White Leghorns) and black, *EE*, (Jersey Black Giants) in his study of shank color, did not have a critical cross in respect to shank color, that would separate the effect of the sex-linked barring genes *Bb* upon black color from a sex-linked gene for shank color. In addition to this, the number of progeny obtained was exceedingly small for a study involving linkage and crossing over percentages. Because of the small number of progeny involved, the percentage of crossing over found by WARREN between the rate of feathering and shank color cannot be differentiated statistically from the crossing over percentage between the rate of feathering and barring. This would be expected if the influence of barring on the general genes for melanic pigment, *EE*, is considered.

This would also apply to the work of SEREBROVSKY and WASSINA (1926), who stated that they found two questionable crossovers among 69 individuals.

HERTWIG (1933) also used crosses which were not critical enough for adequate analysis of shank color. She made the statement that the factor for slow feathering and inhibitor for negroid plumage color and dark mesodermal shank color in the crosses studied were absolutely coupled.

DUNN and JULL (1927) used a cross of White Silkie × White Leghorns in a study of the inheritance of dark shank color. This cross again is not critical enough as it does not differentiate between the effects of the barring gene brought in by the Leghorns (*BBEEII*, *B-EEII*) and the effect that might be due to a pair of sex-linked factors for melanic shank color.

In a more recent publication HAGEDOORN (1930) cites a cross of a White Leghorn male with two Barnevelder females. This would constitute another non-critical cross for shank color, since it is a cross of a *BBEEII* male with a non-barred, non-black female *b-eeii*. HAGEDOORN states that in crosses of Barnevelder male × dark-shanked females, it was shown that the yellow of the Barnevelder was not dominant, as in the case of the Leghorn, but recessive. A more fitting explanation, however, might well be that the yellow shank of the Barnevelder is recessive because it is non-barred (*bb*, *b-*) non-extended black (*ee*) bird, whereas the yellow shanks of the Leghorn are due to the barring gene *B*, which is dominant, thus making it appear that the yellow shanks of the Leghorn are dominant.

HAGEDOORN also states that all of the F_1 progeny obtained from the White Leghorn male × the Barnevelder females had yellow shanks, which was expected. In the F_2 generation, however, some of the females had dark shanks, but all of the males had yellow shanks. He explains this by assuming that the Barnevelder has yellow legs because it lacks a factor termed *BB*, and also lacks the sex-linked factor *AA*, the genetic formula for the color of the Barnevelder's shanks being given as *a-bb*, whereas the White Leghorn male would be *AABB*. It will be noted that this assumption is equivalent to stating that the Barnevelder females are non-barred, non-extended blacks, *b-ee*, whereas the White Leghorn males would be barred, extended blacks, *BBEE*. Both of these latter symbols are preferable to use in the light of the present data. Both formulae, of course, are identical in their action and result.

MACARTHUR (1933) stated, "That on the basis of published data, a great number of uncertainties and inconsistencies in the proposed arrangement of the genes on the sex chromosome map demonstrated the need for correction by further investigations." Such a situation is readily understood when it is realized that MACARTHUR and others in their investigations on crossing over used material involving certain characters the nature of whose inheritance has not been determined by parental crosses constituting a critical test. For instance, such genes as *Dd* (sex-linked dark shank color), *LiLi* (sex-linked down color), and *BrBr* (iris color) have not been satisfactorily explained as to their mode of inheritance so that they might be used in crossover investigations with impunity. To make matters more complicated some investigators have attempted crossover studies with too few progeny.

MACARTHUR (1933) also states that "the breed correlation between black feathers and dark shanks and dark eyes is also broken in many instances." This does not agree with the results obtained in this investigation. The difference is no doubt due to two things; first, the barring of the Silver Campine is not sex-linked as in the barring reported for the Barred Plymouth Rock; second, that the leaden blue shanks of the Silver Campine might indicate a separate gene for melanic shank color which the Barred Plymouth Rock does not have. If there is a separate gene for shank color, MACARTHUR'S statement might hold true for the material that he used but would not apply to the results as reported in this investigation where crosses were used that did not involve separate genes for melanic shank color.

It is evident from the data presented that the black plumage genes, *EE*, are of a general nature and that they cause the deposition of melanic pigment in the shanks as well as in the plumage. It has been shown also that there is as much variation in the effect of the black genes *EE* upon plumage

color as upon the shank color, which is indicated by the standard deviation. It has been demonstrated also that the barring genes *BB*, which are sex-linked, have a restricting effect upon the melanic pigment in the shanks as well as in the plumage. The restricting effect that these genes have upon shank color, however, vary to some extent although this variation is probably no greater than the variation in plumage color found in the F_1 heterozygous black females of the cross of Rhode Island Red males × Barred Plymouth Rock females.

THE EFFECT OF THE PLUMAGE COLOR INHIBITOR GENES *II* UPON MELANIC SHANK COLOR

In reciprocal crosses of Jersey Black Giants with White Leghorns, GODFREY and QUINN (unpublished data, BELTSVILLE RESEARCH CENTER) observed that all of the 134 F_1 progeny from the White Leghorn male × Jersey Black Giant females had yellow shanks free of any melanic pigment. The F_1 progeny from the reciprocal cross, however, consisted of females with slate-colored shanks and males all of which had yellow shanks.

In another cross, using a Black Minorca male × White Leghorn females, it was found that the 64 F_1 males had yellow shanks and of the 51 F_1 females 49 had blue shanks and 2 had yellow shanks. The 2 pullets with yellow shanks are probably the result of some error or mixing of the stock because even if these females lacked melanic pigment their shank color should be white and not yellow, due to the dominance of the *WW* genes for white skin color brought in the cross by the Black Minorca parental stock.

The results from these two crosses involving black plumage birds with dark shanks mated with White Leghorns with yellow shanks can be accounted for by the effect of the barring gene and the inhibiting genes, *II*, of plumage color. In the cross of the White Leghorn male, *BBEEII*, × Jersey Black Giant females, *b-EEii*, the resulting progeny would be of the formulae *BbEEIi* males and *B-EEIi* females. In this case all of the progeny would be expected to have yellow shanks because the barring factor would restrict the melanic pigment in the shank and plumage of both sexes. In the reciprocal cross and in the corresponding cross of the Black Minorca male × White Leghorn females the F_1 male progeny would be *BbEEIi* and should have yellow shank color, which proved to be the case. The female progeny, however, were of the genotype *b-EEIi* and although they were white in plumage color their shanks appeared as a dilute black (slaty or blue colored). This would be expected on the assumption that the melanic pigment gene *E* was present and the restrictor of melanin, *B*, absent in these F_1 females. As these F_1 females had a more dilute color in their shanks than the parental black breeds and the only difference between the genotype of black females with dark shanks, *b-EEii*, and the genotype of the

F_1 females *b-EEIi* is the presence of the inhibitor of plumage color, it is assumed that the difference (dilution of black in the shanks) is due to the inhibitor. Hence, in addition to the effect of barring on melanin in the plumage and the shanks, there appear to be other genes that affect the melanin in the plumage and the shanks, in this case a dilution effect of the melanic shank color by the plumage color inhibitor genes, *II*.

THE EFFECT OF THE CHROMOGEN GENES *cc* UPON DARK SHANK COLOR

There is also some evidence that the genes for lack of chromogen, *cc*, affect the melanic pigment in the shanks in a similar manner to that of the inhibiting genes, *II*. It is known that the White Langshan and the White Minorca have blue shanks. It is also known that these two breeds are reputed to be derived from the Black Langshan and the Black Minorca, respectively (PLATT 1925). The genotypes involved and the shank color in each breed is suggested to be as follows:

Black Langshan, *bbEEiiCC*, non-barred, black, non-inhibitor, and chromogen, black shanks.

Black Minorca, *bbEEiiCC*, same as above, black shanks.

White Langshan, *bbEEiicc*, same as above but lack chromogen, blue shanks.

White Minorca, *bbEEiicc*, same as above, blue shanks.

Thus it appears that the lack of chromogen in certain breeds has a dilution effect upon shank color, which is blue in the case of the *EEcc* birds and black in the *EECC* birds.

Previous investigations have shown that the genes, *WW*, are of a general nature and have the effect of producing white skin, beak and shank color and that the alleles, *ww*, produce yellow color. Crosses have been made and the results reported in this paper which show that the genes *EE* are of a general nature and affect not only the plumage color but also the deposition of melanic pigment in the shanks. In all probability they also affect the beak and eye color. Other crosses have been made which show the modifying effect of the barring genes *BB*, the inhibiting genes, *II*, and the genes, *cc*, for the lack of chromogen upon the deposition of melanic pigment in the shanks. There are probably other modifying genes for black, such as the presence of varying amounts of silver or red, and of hormones, especially sex hormones.

BATESON and PUNNETT (1911) crossed the White Silky × Brown Leghorns, the results indicating sex-linkage of shank color. Because the Brown Leghorns are a sex-linked, non-silver and a non-extended black, which the present data show does not influence melanic pigment in the shanks, it is possible that there is a pair of sex-linked genes for shank color. However, although the Brown Leghorn is reported to be an *ee* bird, it carries a con-

siderable amount of dark color. In the female, this color takes on the nature of a stippling effect of brown with a darker brown, and the male has solid black plumage in the breast and body and various amounts of black in the wings, tail, hackle, and other parts of the body. This extra amount of darker pigmented plumage in an *ee* bird may or may not have some effect aside from the sex-linked melanic shank color upon the presence or absence of melanin in the shanks. Hence, even such a cross as this one may not be critical enough to determine whether or not there is a sex-linked factor for dark shank color. However, until such observations are shown not to be well-founded, the fact that there is strong evidence, as reported by these two workers, for the existence of a sex-linked factor for dark shank color cannot be overlooked.

From all of the data gathered from various sources, including the data in this paper, the writer submits herewith nomenclature for a set of genes for shank color, including a sex-linked shank color gene. The various combinations of genes given in this list is an attempt to explain the inheritance of shank color with various combinations of the genes discussed in this paper. This list is by no means complete since it considers only the simpler effects upon shank color and omits all of the heterozygotes with their attending complications.

KEY TO GENES USED

Genes and Their Alleles

WW non-lipochrome (white color)	*ww* lipochrome (yellow color).
BB sex-linked barring plumage pattern restricts the black plumage to bars and practically a dominant restrictor of melanic pigment in the shanks.	*bb* non-barred plumage pattern, no restriction of melanic pigment in the shanks.
[1]*DD* inhibitor of melanic (dark) shank color. Sex-linked.	*dd* sex-linked gene for melanic pigment in the shanks.
EE extension of black plumage color, and of melanic pigment in the shanks.	*ee* non-extension of black plumage and shank color.
CC chromogen for plumage color non-restrictor of melanic pigment in the shanks.	*cc* lacks chromogen and has a diluting effect on the melanic pigment in the shanks.
II inhibitor of plumage color, dilutes the melanic pigment in the shanks.	*ii* non-inhibitor of plumage color and non-inhibitor of melanic pigment in the shanks.

[1] At present not critically differentiated from the barring gene, *BB*.

The Effect of Varying Combinations of the Above Genes upon Shank Color[2]

Males	Females	
	1. Barring—Black Plumage Series	
WWDDBBEE	*WWD-B-EE*	white shanks (neither lipochrome nor melanic pigment present).
wwDDBBEE	*wwD-B-EE*	yellow shanks (lipochrome, melanic pigment, *EE*, and restrictor, *BB*, all present).
[3]*WWddBBEE*	*WWd-B-EE*	possibly blue shanks (no lipochrome present but melanic pigment, *EE* and *dd*, and restrictor, *BB*, present).
[3]*wwddBBEE*	*wwd-B-EE*	possibly dark or green shanks (lipochrome, melanic pigment, *EE* and *dd*, and restrictor, *BB*, present).
	2. Barring—Non-black Plumage Series	
WWDDBBee	*WWD-B-ee*	white shanks (neither lipochrome nor melanic pigment present).
wwDDBBee	*wwD-B-ee*	yellow shanks (lipochrome pigment present but no melanic pigment present).
[3]*WWddBBee*	*WWd-B-ee*	possibly blue or light blue shanks (no lipochrome but melanic pigment, *dd*, and restrictor, *BB*, present).
[3]*wwddBBee*	*wwd-B-ee*	possibly green or light green shanks (lipochrome, melanic pigment, *dd*, and restrictor, *BB*, all present).
	3. Non-barred—Black Plumage Series	
WWDDbbEE	*WWD-b-EE*	dark shanks (neither lipochrome nor restrictor, *BB*, present but melanic pigment, *EE*, present).
wwDDbbEE	*wwD-b-EE*	dark shanks (lipochrome and melanic pigment, *EE*, present but restrictor, *BB*, absent).
[3]*WWddbbEE*	*WWd-b-EE*	dark shanks (neither lipochrome nor restrictor, *BB*, present but melanic pigment, *EE* and *dd*, present).

[2] All heterozygotes omitted.

[3] Genotypes at present not demonstrated.

[3]*wwddbbEE*	*wwd-b-EE*	dark shanks (lipochrome and melanic pigment, *EE* and *dd*, present and restrictor, *BB*, absent).

4. Non-barred—Non-black Series

WWDDbbee	*WWD-b-ee*	white shanks (no lipochrome, melanin, or restrictor present).
wwDDbbee	*wwD-b-ee*	yellow shanks (lipochrome present; melanic pigment and restrictor absent).
[3]*WWddbbee*	*WWd-b-ee*	dark shanks (blue) (melanic pigment, *dd*, present; lipochrome and restrictor absent).
[3]*wwddbbee*	*wwd-b-ee*	dark shanks (green) (lipochrome and melanic pigment, *dd*, present; restrictor absent).

5. Chromogen Series

A. Non-barred black

WWDDbbEECC	*WWD-b-EECC*	dark shanks (lacks lipochrome; melanic pigment, *EE*, and chromogen present).
wwDDbbEECC	*wwD-b-EECC*	dark shanks (lipochrome, melanic pigment, *EE*, and chromogen present).
WWDDbbEEcc	*WWD-b-EEcc*	blue or slaty blue shanks (lipochrome, restrictor, and chromogen absent; melanic pigment, *EE*, present).
wwDDbbEEcc	*wwD-b-EEcc*	green shanks (chromogen and restrictor absent; melanic pigment, *EE*, and lipochrome present).

B. Barred—Black

[3]*WWDDBBEECC*	*WWD-B-EECC*	white shanks (lacks lipochrome; melanic pigment, *EE*, restrictor, *BB*, and chromogen, *CC*, present).
wwDDBBEECC	*wwD-B-EECC*	yellow shanks (lipochrome, melanic pigment, *EE*, chromogen, *CC*, and restrictor, *BB*, all present).
[3]*WWDDBBEEcc*	*WWD-B-EEcc*	white shanks (as above, except chromogen genes lacking).

wwDDBBEEcc	*wwD-B-EEcc*	yellow shanks (as above, except chromogen genes lacking).

6. Inhibitor of Plumage Color Series

[3]*WWDDBBEEII*	*WWD-B-EEII*	white shanks (lacks lipochrome, with melanic pigment, *EE*, restrictor, *BB*, and inhibitor of plumage pigments *II* present).
wwDDBBEEII	*wwD-B-EEII*	yellow shanks (lipochrome, melanic pigment, *EE*, restrictor, *BB*, and inhibitor of plumage pigments, *II*, all present).
[3]*WWDDbbEEII*	*WWD-b-EEII*	blue shanks (lacks lipochrome and restrictor, *BB*, with melanic pigment *EE*, and inhibitor, *II*, present).
wwDDbbEEII	*wwD-b-EEII*	dark green shanks (restrictor, *BB*, absent; with lipochrome, melanic pigment, *EE*, and inhibitor of plumage pigments present).

SUMMARY

The genes *WW*, *Ww*, or *ww* are presumably present in all breeds and varieties of chickens, although evidence of their presence in the shanks is often masked by the deposition of melanic pigment in the shanks.

There is a single autosomal gene difference between white shank color (the absence of lipochrome pigment, *WW*) and yellow shank color (the presence of lipochrome color, *ww*) the former being dominant to the latter.

Dark blue and light blue shanks are caused by the deposition of melanic pigment in various cells of shanks that have a white skin for a ground color. Dark green and light green (willow) shanks are caused by the deposition of melanic pigment in the shanks which have a yellow skin for a ground color.

Black shanks are caused by the deposition of melanin in the shanks and appear the same in black plumage birds whether they have yellow or white skin color; however, one can be distinguished from the other in respect to skin color by examining the bottom of the feet. In the one case they will be white and in the other yellow.

The black plumage genes *EE* appear to have a general effect upon the deposition of melanic pigment and cause the deposition of melanic pigment in the shanks as well as in the plumage.

Any gene that affects the extension of black pigment in the plumage seems to have a restricting effect upon the melanic pigment in the shanks. This is especially true in the case of the barring genes.

Dark shank color caused by the black plumage genes, *EE*, is not sex-linked. However, it appears so when associated with modifying restrictive factors for the extension of black genes *EE* that are sex-linked, such as the barring factors.

There is still the possibility that there is a sex-linked dark shank color gene which has not thus far been adequately differentiated from the effect of black and barring plumage genes.

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THE INDUCTION BY X-RAYS OF HEREDITARY CHANGES IN MICE

GEORGE D. SNELL[1]

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

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INTRODUCTION

Although numerous experiments have been carried out, both before and since the classical work of MULLER with Drosophila, to determine if X-rays induce hereditary changes in mammals, the results heretofore obtained have all been either negative or, when considered individually, decidedly inconclusive. The production of sterility in the X-rayed individuals has been proved (ALBERS-SCHÖNBERG 1903, BAGG and LITTLE 1924, DOBROVOLSKAÏA-ZAVADSKAÏA 1928, SNELL 1933a, and many others); so also has the occurrence of defective embryos (indicated in some cases only by the reduction in number of viable young) in litters conceived between raying and the onset of sterility (REGAUD and DUBREUIL 1908, MARTIUS and FRANKEN 1926, YAMAMOTO 1929, MURPHY 1930, STRANDSKOV 1932, SNELL 1933a); moreover, at least two investigations (MARTIUS and FRANKEN 1926, and MARTIUS 1927 describing the work of SCHUGT and KIKKAWA) have indicated the production of complete sterility and of subnormal growth in some or all F_1 individuals when both parents, or when the female parents alone, were rayed, this last perhaps due to faulty nutrition of young whose mothers had been "burned" by the X-rays (NÜRNBERGER 1926). The occurrence, however, of any kind of heritable variation as a result of the treatment has remained uncertain. BAGG and LITTLE (1924, see also BAGG 1925, and later papers by the same authors), in pioneer studies on X-ray induction of mutations, reported the appearance in the third and subsequent generations of the descendants of two different pairs of X-rayed mice of a recessive mutation irregularly affecting eyes, feet, and viscera. In the experiments of DOBROVOLSKAÏA-ZAVADSKAÏA (1928), the well known dominant mutation "tailless" (or short-tailed) appeared several times among the first and second generation progeny of treated male mice. This experiment also yielded an F_1 male the top of whose cranium was unhardened, an F_2 male with one digit of a front foot missing (both these variants failing to survive), and an F_2 male showing a nervous motion of the head. This last variation and the tailless mutation were perpetuated. STRANDSKOV (1932) found one male with a duplicated

[1] The investigations described in this paper were nearly all carried out while the writer was NATIONAL RESEARCH COUNCIL Fellow at the UNIVERSITY OF TEXAS.

penis in the second generation following treatment of male guinea-pigs. No similar variation appeared in the control. Considered together, these three experiments seem to indicate that an effect on offspring results from the X-ray treatment; analysed separately, with due allowance for the nature of the control, statistically significant proof of induced genetic changes is found to be lacking.

Since the results of former investigations proved inconclusive, it appeared of interest following the discovery by MULLER of X-ray mutations in Drosophila to re-investigate the possibility of inducing hereditary changes in mammals through the application of X-rays. Such a re-investigation was under-taken by the writer in 1931. An analysis of the effect of X-rays on the fertility of treated males has already been published (SNELL 1933a). A description of the technique used in raying is given in this earlier paper, and will not be repeated here. A preliminary report of the high rate of induced change appearing in later generations of mice has also been given (SNELL 1933b), together with detailed accounts of some of these changes (SNELL, BODEMANN, and HOLLANDER 1934; SNELL and PICKEN, in press). This paper presents in detail the evidence that approximately one third of the offspring of males rayed with doses in the neighborhood of 600 r-units carry induced translocations.

For a more complete bibliography of the subject than is here given, reference may be had to papers by DOBROVOLSKAÏA-ZAVADSKAÏA (1928), NÜRNBERGER (1927 and 1930), and HERTWIG (1932a).

STOCKS AND GENETIC TECHNIQUE

Since early investigations of the genetic effects of X-rays on Drosophila indicated a high rate of production of recessive mutations, and particularly of recessive lethals, the experiments with mice were planned with a view to revealing these types of genetic changes. A careful survey was made of the inbred stocks of mice available for work of this sort in different genetics laboratories, and of the different systems of matings which might be used to reveal induced mutations. The aim was to set up the experiment in such fashion as to give the greatest chance of discovering any mutations that might be induced with the least use of pens and of time; or more concisely, to give the maximum probability of mutation detection per pen per week.

The P_1

Five stocks of mice were finally selected for the experiment, as follows:

1. The *R*-stock was an inbred stock of mice homozygous for five recessive mutant genes, *a*, *b*, *d*, s_e, and *p*. As *d* and s_e are very closely linked these five genes served to mark four chromosomes. The stock was fur-

nished by Prof. WILLIAM H. GATES. All the X-rayed males were from this stock.

2. The *L*-stock was an inbred stock homozygous for one recessive mutant gene, *a*. It was obtained from Dr. L. C. STRONG of the ROSCOE B. JACKSON MEMORIAL LABORATORY. It was characterized by high fertility and a high degree of uniformity. Approximately one half of the original untreated parent females came from the stock.

3. The *Ag*-stock was an inbred stock also supplied through the kindness of the ROSCOE B. JACKSON MEMORIAL LABORATORY. It was homozygous for the wild-type genes, except that some individuals carried *a*.

4. The *A*-stock was an inbred strain homozygous for A^w and c^{ch}. It was supplied by Dr. GREGORY PINCUS.

5. The *F*-stock consisted of the first generation progeny from a cross between the *Ag*-stock and the *A*-stock. It was characterized by a con-

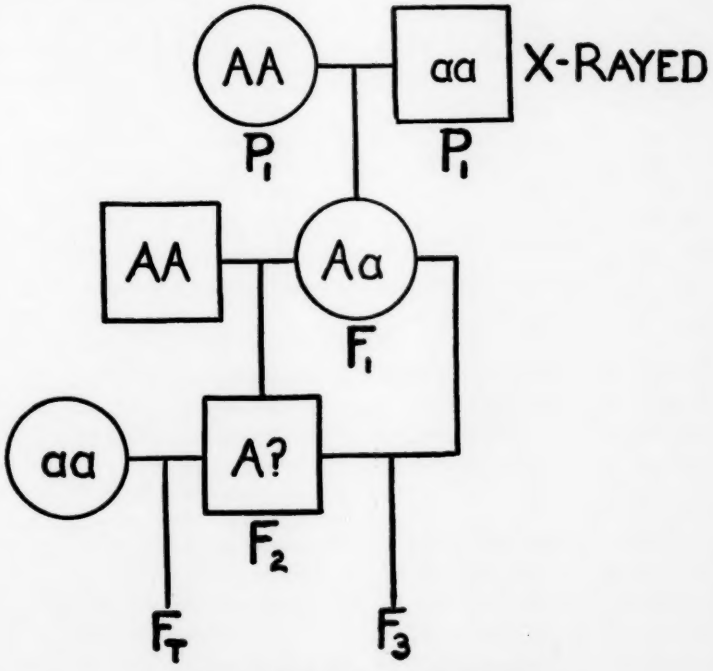

FIGURE 1.—Diagram showing system of mating.

siderable degree of hybrid vigor, the females breeding well and producing large litters. Tumor incidence was rather high in old females, however.

All of the original untreated female parents not from the *L*-stock were from this stock.

The system of matings used is outlined in figure 1. For simplicity, only one of the marker genes involved in the cross is indicated in the figure.

The F_1

Males of the *R*-stock were mated to females of the *L*-and *F*-stocks, the offspring of these matings being designated as the F_1. Each *R*-stock male was mated once before raying to furnish the control, then several times during the one or two weeks fertile period following raying to produce F_1 test litters. The details as to the method of timing matings, the duration of the fertile period following raying, and the effect on F_1 litter size have been described in a previous paper (SNELL 1933a) and will not be repeated here. In subsequent generations the treatment of the test and control groups was identical. The pens occupied by individuals of the two groups were selected at random so that there could be no significant difference in location or in feeding. Moreover, the records were so kept that it was not known when a given litter was examined whether it belonged to the test or to the control.

The F_2

The F_2 was produced, not by mating F_1 individuals *inter se*, but by mating them to mice from the *L*-, *F*-, or *Ag*-stocks. With a few exceptions, F_1 mice from *F*-stock females were mated back to the *F*- or *Ag*-stocks, F_1 mice from *L*-stock females to the *L*-stock. The phenotype of the F_2 was thus like that of the P_1 females, but most of them carried one or more recessive marker genes.

The F_T

To test F_2 individuals for recessives, they were mated to *R*-stock individuals, and, with a few exceptions, at least five offspring reared until they were old enough so that their phenotypes could be determined (usually about ten days). This composed the F_T generation.

The F_3

The F_3 was produced by mating F_2 individuals back to their F_1 parents. Where the F_1 was a male, as many as three daughters were often backcrossed; where the F_1 was a female, usually only one son was backcrossed. The average number backcrossed in each case is shown in table 5 in the column headed "Mean number of F_2 mated to each F_1." The phenotype of F_3 individuals was determined by one or more examinations made between the first and the fourth weeks of their age. At about four weeks of age, they were killed, and an autopsy performed to detect possible abnormalities of the internal organs.

With a few exceptions, all pregnant females were isolated in separate, freshly cleaned pens, and examined daily until the birth of the litter.

The above system of matings was used because calculations showed it to be better adapted than any other for the detection of lethal and visible mutations.

The detection of recessive lethal mutations depends on the alteration of ratios in the F_3 generation. If a lethal occurs on a marked chromosome in the germ tract of a P_1 male, an F_1 individual inheriting it will be of the genotype AL/al, where l is the lethal mutation and a the marker gene. Assuming complete linkage, one half of the F_2 individuals will be of the genotype AL/al like their parent. Such heterozygous individuals can be distinguished from their homozygous sibs by the test mating. When backcrossed to their F_1 parent, the lethal which they carry will prevent the appearance of F_3 individuals homozygous for the recessive marker gene, as all such individuals will be homozygous for the lethal also and will die. Hence the failure of a recessive to appear following the backcross of an F_2 individual, proved by the test mating to carry the recessive, is evidence for the presence of a linked lethal. Twenty offspring from such a mating, if all of them show the dominant phenotype, are sufficient to establish a strong presumption that a lethal is present, while if no lethal is linked with the marker gene, one litter is usually sufficient to show it. By no other system of matings can the presence or absence of lethals in mice be so easily determined. Even with this method, however, only a fraction of all treated chromosomes carried by F_1 individuals are tested, the maximum being four (the number of marked chromosomes) out of twenty (the haploid number), and the proportion actually realized considerably less than this.

In the case of recessive visible mutations, over fifty percent of all treated chromosomes carried by F_1 individuals are tested by the system of matings used. In previous attempts to detect induced mutations in mammals, F_2 individuals have been mated together to produce the F_3. Our method of backcrossing F_2 individuals to their F_1 parents gives just double the chance of detecting visible mutations per F_1 individual. The practice of backcrossing from one to three F_2 individuals to each F_1 was adopted because calculations showed the use of larger numbers to be subject to diminishing returns. Three daughters of a single F_1 male will, on the average, carry seven-eighths of all his treated chromosomes. These three daughters can be backcrossed in a single pen. The inclusion of a fourth daughter would necessitate the use of a second pen, and would increase the fraction of the treated chromosomes available for testing by only one-sixteenth. The number of F_3 individuals raised from each backcross was determined by similar considerations, the actual figures being

given in table 5 in the column headed "Mean number of autopsied F_3 mice per F_2." As has been pointed out by PAULA HERTWIG (1932b), previous investigators have, in some cases, raised a very inadequate number of F_3 litters.

The autopsy that was performed consisted of a standardized examination of salivary glands, thyroid, trachea, heart, lungs, thymus, digestive organs, kidneys, testes and ovaries and their ducts, the accessory glands of the reproductive system, and parts of the skeletal and circulatory systems. It was undertaken in the belief that, because of the relatively simple external anatomy but complex internal anatomy of mammals, many mutations may not be externally visible. The belief was substantiated by the results; the only mutation found affects primarily the shape of the spleen, and would not have been detected in the absence of the autopsy.

While the experiment was not originally planned with the detection of induced translocations in mind, results obtained by Dr. H. B. GLASS at the UNIVERSITY OF TEXAS with translocations in Drosophila soon suggested the possibility of detecting them in mice by watching for F_1 individuals that consistently produced small litters. The practice followed throughout the experiment of outcrossing F_1's to mice from untreated stocks made it easier to discover such individuals than would have been the case if the F_1's had been mated *inter se*. By good fortune the system of matings used in the experiment was thus as well adapted to the detection of translocations as to the detection of visible and lethal mutations.

EVIDENCE FOR THE PRODUCTION OF TRANSLOCATIONS

The presence among the F_1 test mice of a considerable number of individuals that consistently produced small litters is indicated by table 1, which shows the frequency distribution of F_1 mice with respect to the mean size of the F_2 litters which each produced. From many of the F_1 mice only a single F_2 litter was obtained, from others six or seven or more, the average being 1.8 F_2 litters per F_1 mouse. The position of many of the

TABLE 1

Frequency distribution of F_1 mice with respect to the mean size of the F_2 litters produced by each F_1 mouse.

MEAN SIZE OF F_2 LITTERS BY EACH F_1 MOUSE	1	2	3	4	5	6	7	8	9	10	11	12	TOTAL
Number of F_1 test ♂♂	1	2	5	8	2	7	10	10	13	1			59
Number of F_1 test ♀♀	3	5	4	6	2	3	3	11	15	2	1		55
Number of F_1 control ♂♂					2	7	12	8	9	5	2	1	46
Number of F_1 control ♀♀					4	12	10	15	9	4	3		57

F_1 mice in the frequency distribution is thus determined by averaging the number of young in several F_2 litters. It will be seen from the table that whereas none of the 103 F_1 control mice produced litters averaging less than five, 34 of the 114 F_1 test mice produced litters averaging from one to four. The distribution of the controls is unimodal, with the mode at eight; the distribution of the test animals is strikingly bimodal, with modes at four and nine. Thus there is clearly a tendency for some of the test animals to produce small litters. These animals will be referred to as "semi-sterile."

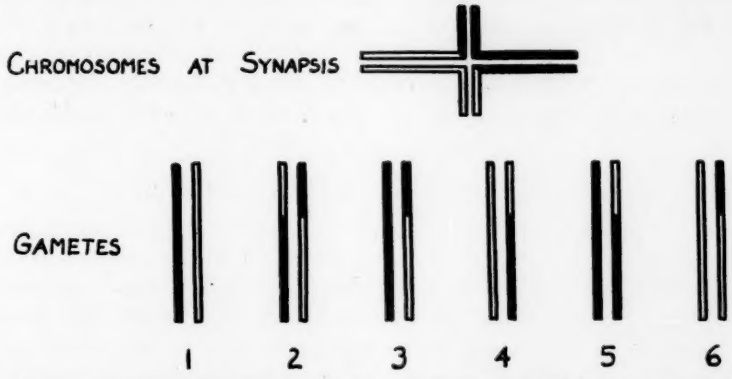

FIGURE 2.—Diagram showing the types of gametes formed by an individual heterozygous for a reciprocal translocation (based on translocations involving the second and third chromosomes of *Drosophila melanogaster* as studied by GLASS, and by DOBZHANSKY and STURTEVANT). Type 1 is entirely normal. Type 2, when combined with a normal gamete, gives an individual heterozygous for the translocation like the heterozygous parent. The chromosomal balance of such individuals is normal. Types 1 and 2 are formed with equal frequency, and together make up at least 50 percent of the total. Types 5 and 6, in the case of certain translocations in Drosophila, are not formed at all. Types 3, 4, 5, and 6, when combined with normal gametes, give zygotes with chromosomal unbalance, usually non-viable. Simple translocations in Drosophila likewise produce a certain proportion of gametes with chromosomal unbalance. Whether the translocations in mice herewith described are simple or reciprocal, and whether they produce two or four types of gametes with chromosomal unbalance, is undetermined.

Ten of the semi-sterile F_1 animals (♀♀ $F_1$109, $F_1$99, $F_1$145; ♂♂ $F_1$93, $F_1$107, $F_1$146, $F_1$262, $F_1$271, $F_1$285, $F_1$292) were saved for further study. In an analysis of the descendants of one of them, ♂ $F_1$146, SNELL, BODEMANN, and HOLLANDER (1934) have shown that the semi-sterility is due to the presence of a translocation. Matings between $F_1$146 and normal females produced normal mice, semi-sterile mice, and abnormal embryos approximately in the ratio 29: 29: 42. Similar ratios were produced by semi-sterile sons of ♂ $F_1$146. The abnormal embryos usually do not come to term, and hence account for the small size of the F_2 litters. Their oc-

currence when semi-sterile mice are outcrossed to normal mice from untreated stocks rules out the possibility that they are due to the segregation of a recessive lethal gene. All the facts are in accord with the idea that semi-sterile mice in the $F_1$146-stock are heterozygous for a translocation, and that the small litter size is due to the formation of zygotes which are non-viable because they have inherited unbalanced chromosome combinations. The types of gametes probably formed by individuals heterozygous for the translocation are indicated in figure 2.

While none of the other semi-sterile stocks has been tested as thoroughly as the $F_1$146 stock, considerable data are available in regard to the remaining nine of those selected for intensive study. Of these, the $F_1$271-stock is perhaps the best tested, and is of particular interest because the translocation appears to be linked with the recessive marker gene, "brown" (*b*). The evidence is summarized in table 2. All the mice listed in the first

TABLE 2

Descendants of ♂ $F_1$271 showing probable linkage between semi-sterility and the gene for brown (b).

COLONY NUMBER	GENOTYPE	MOTHER'S COLONY NUMBER	FATHER'S COLONY NUMBER	NUMBER OF LITTERS	MEAN SIZE OF LITTERS
(a) Probably semi-sterile					
♀ $F_2$295	*AaBbDS$_e$/ds$_e$*	*Ag*94 *BB*	$F_1$271 *Bb*	1	3
♀ $F_2$719	*AaBb*	*Ag*75 *BB*	"	3	3
♀ *F$_T$*3698	*aabbds$_e$/ds$_e$*	*R*343 *bb*	"	2	3
♂ *F$_T$*3770	*aabbds$_e$/ds$_e$Pp*	*R*342 *bb*	"	5	3.2
♂ *F$_T$*3771	*A^{W}abbDS$_e$/ds$_e$Pp*	"	"	4	3.5
♂ *N$_8$*7	*aaBbNnWw**	*N$_4$*378 *BB*	*F$_T$*3770 *bb*	2	2.5
♀ *N$_8$*166	*aabb*	*R*477 *bb*	*N$_8$*7 *Bb*	1	4
♀ *N$_8$*167	*aabbds$_e$/ds$_e$*	"	"	1	3
(b) Probably normal					
♀ $F_2$720	*AaBBDS$_e$/ds$_e$*	*Ag*75 *BB*	*F$_1$*271 *Bb*	4	6.7
♀ *F$_T$*3697	*AaBbDS$_e$/ds$_e$Pp*	*R*343 *bb*	"	1	6
♀ *N$_8$*0	*A?BBNnWw*	$F_2$719 *Bb*	*N$_4$*413 *BB*	2	9.5
♀ *N$_8$*1	*aaB?NnWw†*	"	"	2	11
♀ *N$_8$*2	*A?B?nnww†*	"	"	1	12
♀ *N$_8$*4	*aaBbNnww**	*N$_4$*378 *BB*	*F$_T$*3770 *bb*	2	10
♂ *N$_8$*17	*aaBbNnWw**	*N$_4$*376 *BB*	"	1	11
♀ *N$_8$*23	*aaBbnnww**	*N$_8$*405 *BB*	"	1	9
♀ *N$_8$*24	*aaBbnnWw**	"	"	1	7

* Gene for brown (*b*) may have been derived from untreated stock.

† Not tested for presence of gene for brown (*b*).

column of table 2 are derived from matings between semi-sterile individuals of the $F_1$271-stock and normal individuals of untreated stocks. It will be seen that they fall into two classes, the first producing litters

averaging four or less than four young, the second producing litters averaging six or more than six young. The first class is listed as "probably semi-sterile," the second as "probably normal." There are 8 individuals in the first class, 9 in the second, a good approximation to the 1:1 ratio expected on the hypothesis that semi-sterility is due to the segregation of a translocation. The genotypes of the individuals in question are given in the second column, and their parents' colony numbers and genotypes with respect to brown in the next two columns. In five cases the individuals were not tested for the presence of the gene for brown; in two cases the mating which produced them was such that the gene for brown might have been derived from an untreated stock. The significant data come from the remaining ten cases. In seven of these ten the treated chromosome bearing the gene for brown has been inherited, and in all seven the individual is probably semi-sterile; in the remaining three the untreated chromosome bearing the gene for non-brown (*B*) has been inherited, and in all three the individual is probably normal. A linkage is thus indicated, the odds against the treated chromosome segregating with semi-sterility due to chance along in all of the ten cases being 1023 to 1. The fact that the semi-sterility of some of the ten individuals was only tested by a single litter, and the possibility that ♂$F_1$271 actually carried more than one X-ray-induced translocation, somewhat reduce the presumption of linkage, but the evidence for linkage may be regarded as quite strong even if not entirely conclusive.

Data derived from other semi-sterile stocks all point to the same interpretation of semi-sterility as that given in the case of the $F_1$146- and the $F_1$271-stocks.

In the first place, all of the semi-sterile F_1's so far tested appear to transmit the tendency to produce small litters to a part of their descendants. An incomplete presentation of the data showing this is given in tables 3 and 4, in which are listed all F_1 mice suspected of semi-sterility. In the last column of each table are listed the average sizes of the F_T litters produced by each F_2 mouse from each of the semi-sterile stocks. The number in parenthesis is the number of litters on which each average is based. It will be seen that in many of the stocks some of the F_2 mice exhibit the same tendency to produce small litters that was characteristic of their semi-sterile parents. Thus four F_2 females from ♂$F_1$107 produced litters averaging 2, 3.5, 4, and 7 young. The first three were probably semi-sterile. Some further data pointing in the same direction will be presented in a paper by Miss ELSIE BODEMANN. When all the data are considered, there can be no reasonable doubt that the tendency to produce small litters possessed by all genuinely semi-sterile mice is hereditary.

In the second place, all ten of the specially tested semi-sterile stocks

produce abnormal embryos, many of which are similar in type to the abnormal embryos found in the $F_1$146-stock. These abnormal embryos appear in all cases not only when two semi-sterile individuals are mated together, but also when semi-sterile individuals are mated to individuals from normal, untreated stocks. The data will be presented in a paper by Miss ELSIE BODEMANN. These facts are in accord with the assumption that

TABLE 3

Tabulation of evidence concerning all F_1 males suspected of semi-sterility.

F_1♂	X-RAY DOSE	NUMBER F_2 MICE	NUMBER F_2 LITTERS	MEAN SIZE F_2 LITTERS	NUMBER F_2 MICE	NUMBER F_2 LITTERS	MEAN SIZE F_2 LITTERS	MEAN SIZE ALL LITTERS	CORRECTED MEAN SIZE	P	ABNORMAL EMBRYOS	AVERAGE SIZE OF F_T LITTERS PRODUCED BY EACH F_2 FEMALE†
(a) Control												
352		10	1	10	27	5*	5.4	6.2	6.7	.02		6(1) 8(1)
354		11	2	5.5	9	1	9	6.7		.19		8(1)
151		8	1	8	26	4	6.5	6.8		.13		7(2)
152		10	2	5	38	5*	7.6	6.9	7.2	.08		
(b) Test												
261	800	1	1	1	—	—	—	1.0		<.01		7(1)
116	400	7	4	1.7	1	1	1	1.6		<.01		8(1)
139	600	4	2	2	—	—	—	2.0		<.01		3(1)
285	800	6	2	3	17	7*	2.4	2.6	2.6	<.01	yes	3.5(2) 8(2)
262	800	9	3	3	5	2	2.5	2.8		<.01	yes	5(3) 2.4(5) 4(2)
271	800	12	3	4	2	2	1	2.8		<.01	yes	3(1) 3(2) 6.7(4)
119	600	15	5	3	4	1	4	3.2		<.01		2(1) 3(1)
93	600	18	6	3	9	2	4.5	3.4		<.01	yes	6(1) 6.3(3) 9(2)
107	400	11	4	2.7	52	15*	3.5	3.3	4.0	<.01	yes	2(2) 3.5(2) 4(1) 7(1)
256	800	8	2	4	20	6	3.3	3.5		<.01		3.3(3) 7(1) 8(1)
292	800	8	2	4	6	2	3	3.5		<.01	yes	3(2) 7(1) 8(1)
112	400	16	4	4	9	3	3	3.6		<.01		4(1) 8(1)
146	600	26	7	3.7	18	5	3.6	3.7		<.01	yes	Nine semi-sterile out of eighteen tested F_2
98	400	13	3	4.3	26	6*	4.3	4.3	4.4	<.01		6(1) 9(1)
288	800	8	1	8	1	1	1	4.5		.02		
240	800	13	3	4.3	40	6	6.7	4.8		<.01		6(1) 7(1) 9(1)
180	600	7	1	7	27	6	4.5	4.9		<.01		4(1)
230	800	15	3	5	8	1	8	5.7		.02		2(1) 10(2)
294	1200	13	2	6.5	10	2	5	5.7		.02		6(1) 8(1) 8(1)
53	800	6	1	6	—	—	—	6.0		.29		
281	800	8	1	8	17	3	5.7	6.2		.07		2(1)5(1)
280	800	11	2	5.5	8	1	8	6.3		.13		5.5(2) 6(1) 10(1)
195	600	7	1	7	25	4*	6.2	6.4	7.2	.05		4(1) 7(1)
158	600	8	1	8	69	8	8.6	8.6		>.9	yes	4.5(2) 5(1)

* One or more litters not recorded at birth.

† Average size of all F_T litters produced by F_2 control ♀ ♀ = 6.93.

a semi-sterile mouse gives small litters because it is heterozygous for a translocation.

The incidence of translocations among the F_1 mice from treated sires is surprisingly high. Approximately one-third of all F_1's in the test group are thus affected. The evidence indicating this high incidence is presented in tables 3 and 4.

TABLE 4

Tabulation of evidence concerning all F_1 females suspected of semi-sterility.

F_1 ♀	X-RAY DOSE	NUMBER F_2 MICE	NUMBER F_2 LITTERS	MEAN SIZE F_2 LITTERS	NUMBER F_3 MICE	NUMBER F_3 LITTERS	MEAN SIZE F_3 LITTERS	MEAN SIZE ALL LITTERS	CORRECTED MEAN SIZE	P	ABNORMAL EMBRYOS	AVERAGE SIZE OF F_T LITTERS PRODUCED BY EACH F_2 MALE†
(a) Control												
55		5	1	5	3	2	1.5	2.7		.01		6(1) 7(1)
89		8	1	8	7	4	1.7	3.0		<.01		8.5(2)
124		9	2	4.5	21	7	3	3.3		<.01	no	4.1(9)
130		6	1	6	10	3*	3.3	4.0	5.0	.02		7(2)
47		13	2	6.5	9	3	3	4.4		.04		11(1)
87		9	1	9	9	3*	3	4.5	9.0	.08		9(1)
80		5	1	5	9	2	4.5	4.7		.16		8(1)
(b) Test												
120	600	1	1	1	—	—	—	1.0		.03		
238	800	3	3	1	—	—	—	1.0		<.01		
257	800	1	1	1	—	—	—	1.0		.03		
184	800	2	1	2	4	3	1.3	1.5		<.01		1.5(4)
241	800	4	2*	2	—	—	—	2.0	3.0	.01		
270	800	4	2	2	—	—	—	2.0		.01		
99	400	7	2	3.5	4	3	1.3	2.2		<.01	yes	2.7(6) 5.5(2) 7.5(2)
145	600	3	1	3	7	4*	1.7	2.0	2.2	<.01	yes	4.5(2) 5(2)
231	800	3	1	3	6	3	2	2.2		<.01		5.2(4) 7.3(3)
251	800	7	3	2.3	—	—	—	2.3		<.01		
101	400	2	1	2	6	2	3	2.7		.01		5.2(4)
237	800	4	1	4	7	3*	2.3	2.7	3.0	<.01		8.7(3)
216	800	3	1	3	6	2*	3	3.0	3.5	.02		6.5(4)
97	400	8	2	4	2	1*	2	3.3	4.0	.03		3(1) 5(3)
242	800	4	1	4	3	1	3	3.5		.08		7(1)
109	600	6	1	6	16	5	3.2	3.7		<.01	yes	4.3(7) 6.4(5)
94	600	6	1	6	16	5*	3.2	3.7	3.8	<.01		.
289	800	8	2	4	—	—	—	4.0		.13		
100	400	9	1	9	25	7	3.6	4.2		<.01		5(1)
154	600	15	3	5	7	2	3.5	4.4		.04		5.5(2)
219	800	4	1	4	10	2*	5	4.7	4.0	.16		1.7(3) 5.5(2)

* One or more of litters not recorded at birth.

† Average size of all F_T litters produced by F_2 control ♂♂ = 6.74.

These tables give all F_1 animals, both test and control, which, by virtue of the small size of the litters they produced, may be suspected of semi-

sterility. The first column in each table gives the colony number of the F_1 animal. The second column gives the X-ray dosage in r-units applied to the fathers of the treated group. The next eight columns give data on litter size. The last of these, headed "Corrected mean size," gives the mean size of all the F_2 and F_3 litters of each F_1 mouse which were observed and recorded within 24 hours of birth; all litters recorded more than 24 hours after birth are omitted. Such litters are often depleted when finally examined, and, when included, tend to give a mean litter size that is too low. The next column, headed "P", gives the probability that the difference between the mean size of the F_2 and F_3 litters produced by each F_1 (from the column headed "Mean size of all litters"), and the mean size of the F_2 and F_3 litters produced by all control F_1 of the same sex, could occur by chance alone. The means used are the uncorrected means, including all litters whether recorded at birth or some days thereafter. P is calculated by the method for the difference of two means given by R. A. FISHER (1930). The next column, "Abnormal embryos," shows which animals were used for embryological studies, and which of these gave embryos with open brains, found to be typical of translocation stocks. ($\sigma^{\prime}F_1$292 gave embryos of a slightly different but related type.) I am indebted to Miss ELSIE BODEMANN for the data in this column. The last column, as explained above, indicates the incidence of semi-sterility among the F_2 mice derived from each F_1.

Table 3 contains all F_1 males whose combined F_2 and F_3 litters averaged less than 7 young. For the control group as a whole, the average size of the combined F_2 and F_3 litters was $8.48 \pm .18$, so that an average of 7 may be taken, more or less arbitrarily, as suggesting semi-sterility. Four males from the control group produced litters averaging less than this. However, it is only in the case of male $F_1$352, whose 6 litters averaged 6.2 young, that the difference is significant ($P = .02$). Moreover, the significant difference in this one case, if not due to chance alone, apparently can be explained by the fact that 3 of the F_3 litters were not recorded at birth, and probably had been somewhat depleted by the time they were first examined. With these 3 litters omitted, the average size of the remaining F_2 and F_3 litters becomes 6.7, a figure that does not differ significantly from 8.48. It may be concluded that none of the F_1 control males were semi-sterile.

Turning to the consideration of the F_1 test males, we find that in the case of 23 of them, the combined F_2 and F_3 litters averaged less than 7 young. Moreover, in the case of 19 of these 23, the difference between this average and the average for all F_2 and F_3 litters from F_1 males of the control group (8.48), is very probably significant ($P \leqq .02$), so that the individuals in question may be accepted as semi-sterile. The remaining test

males, listed in column 1 of table 3, require further consideration. Male $F_1$53 produced only one litter, an F_2 litter containing 6 individuals. With these meagre data, no safe conclusion about this male can be drawn. Male $F_1$281 produced 4 litters averaging 6.2 young. $P = .07$. Two daughters produced F_T litters of 2 and 5 respectively, suggesting that one of them, at least, was semi-sterile. On the strength of this, we may assume that ♂$F_1$281 was himself probably semi-sterile. $F_1$280 was a border-line case, with little suggestion of semi-sterility coming from one daughter who produced two F_T litters averaging 5.5 young. Male $F_1$195 was probably normal, his apparent semi-sterility being attributable to the inclusion of one litter not recorded at birth. Male $F_1$158 was not detectably semi-sterile, but he produced occasional abnormal embryos and abnormal young at term, and evidence now in press indicates that he carried a translocation causing the formation of a few defective zygotes, but not enough to effect litter size appreciably. He is therefore included with the semi-sterile animals, though not semi-sterile himself. Assuming ♂♂$F_1$53, $F_1$280, and $F_1$195 to be normal, we arrive at 21 (36.5 percent) as a probable figure for the number of F_1 test animals carrying translocations. Some of these males undoubtedly carried more than one translocation. This is indicated by the high degree of semi-sterility exhibited by some of them, and, in the case of ♂$F_1$93 (unpublished data obtained by Mr. WILLARD HOLLANDER), by the large proportion of semi-sterile mice among his offspring.

The incidence of semi-sterility is not quite so easily determined in the case of the F_1 females. Apparently some of them produced small litters due to causes other than the presence of a translocation. The pertinent data are presented in table 4, which includes all F_1 females whose combined F_2 and F_3 litters averaged less than 5 young. Of the control females, 7 fall within this category, and in the case of 5 of them the difference between the mean size of the litters which they produced and the mean size ($6.96 \pm .21$) of all F_2 and F_3 litters from F_1 control females is probably significant ($P \leqq .04$). Female $F_1$47 may be dismissed as a border-line case, the small size of whose litters is probably due to chance alone. The apparent semi-sterility of ♀$F_1$130 is probably attributable to the inclusion of two litters not examined at birth. With these litters excluded, the mean size of the remaining litters becomes 5.0. Females $F_1$55, $F_1$89, and $F_1$124, however, definitely show a subnormal fertility. Moreover, in the case of ♀$F_1$124, there is some evidence that semi-sterility was transmitted to a son (♂$F_2$466). The 9 F_T litters produced by this son averaged 4.1 young (or omitting two litters not recorded at birth, 4.9 young). On the other hand, five litters of embryos obtained from ♂$F_2$466 contained 38 normals, 4 solid moles, and 1 dead embryo showing a distended and twisted central

nervous system (data kindly furnished by Miss ELSIE BODEMANN). The absence of embryos with the brain deformities characteristically produced by chromosome unbalance, and the large proportion of normals, strongly argue against the conclusion that ♂$F_2$466 carried a translocation. The cases of ♀ ♀ $F_1$55, $F_1$89, and $F_1$124 must be left somewhat uncertain, but much the most probable interpretation appears to be that they produced small litters because of poor health or some abnormal physiological condition, rather than because they had inherited translocations, or a recessive lethal may have been involved, particularly in the case of $F_1$89.

Turning to the F_1 test females, it will be seen (table 4) that there are 21 the mean size of whose litters averaged less than 5 young. Most of these are undoubtedly semi-sterile, but considerable uncertainty attaches to the last six listed in the table excluding ♀ ♀ $F_1$109 and $F_1$94. Female $F_1$154 was very likely normal. The mean size of the litters she produced is 4.4, compared with a mean size of 6.96 for all F_2 and F_3 control litters from F_1 females. The difference between these figures could occur one time in twenty-five due to chance alone ($P = .04$), and since there were fifty-five test females, equivalent differences would be expected to occur one to several times without specific cause. There is, moreover, no evidence that female $F_1$154 produced abnormal embryos, and her one tested son since he produced litters averaging 5.5 young, was quite probably normal. Similar arguments apply to ♀ ♀ $F_1$97, $F_1$242, $F_1$289, and $F_1$100, though the suspicion of semi-sterility in these cases is somewhat greater than in the case of $F_1$154. Female $F_1$219 would be taken for normal were it not for one apparently semi-sterile son. Perhaps as reasonable a conclusion as any is to assume semi-sterility for two of the six doubtful females. This leaves a total of 17 semi-sterile females. Hence approximately 17, or 30.9 percent, of the F_1 test females carry translocations.

Combining the figures for males and females, we find that approximately 38 mice, or 33.3 percent of the total carry one or more X-ray induced translocations. This may be compared with the figure for *Drosophila melanogaster* reported by MULLER and ALTENBURG (1930) of 117 translocations in 883 flies from X-rayed males (13.3 percent), and the figure for corn indicated by the investigations of STADLER (1931) of about 25 percent following treatment of mature pollen, the dose in each case being roughly determined by the maximum tolerance of the species.

In addition to the semi-sterile mice, there were a number which proved to be completely sterile, giving no litters at all. Six were F_1 test males. These males were mated with (put in the same pen with) one or more normal females, usually for periods of three or more weeks. Most of the females had been, or were later, proved fertile by matings to other males.

No pregnancies resulted. One F_1 test female (F_1241) was likewise proved sterile. One control male and two control females also failed to give young, but they were not so thoroughly tested as the above-mentioned test animals, the male particularly having been mated to only one female, so there is some reason to doubt if they were actually sterile.

The seven sterile animals in the X-rayed group may be interpreted as extreme cases of semi-sterility. This interpretation is particularly plausible in the case of ♀ F_1241. Two proven semi-sterile females, F_1120 and F_1257, verged on complete sterility, giving one litter of one in five matings, and one litter of one in three matings, respectively. At least two different males were used in each case. Female F_1241 may well have been merely slightly more "semi-sterile" than these two. The interpretation does not fit so well in the case of the males. Only one semi-sterile male, F_1261, verged on complete sterility. This male gave one litter of one. He died at three and one half months of age before very thorough tests of his fertility had been made. As F_1 males could be mated to two or three females at once, and shifted frequently from one pen of females to another, it was usually possible to obtain several litters even from those with the lowest fertility, provided they were fertile at all. There is thus some reason to believe that the six above mentioned test males were truly sterile. However, the above interpretation may be the correct one in the case of some or all of the seven sterile F_1 test animals. If so, the figure of 33.3 percent for the incidence of semi-sterility in the test group is conservative.

Another explanation, at once plausible and interesting, is that the six sterile F_1 test males were sterile because they had inherited a Y chromosome which had been fragmented or deleted by the X-ray. Male Drosophila lacking a Y chromosome are viable but sterile. *A priori*, we might expect the same to be true of male mice.

ABNORMALITIES OF DEVELOPMENT ATTRIBUTABLE TO THE TRANSLOCATIONS

Studies by SNELL, BODEMANN, and HOLLANDER (1934,) SNELL and PICKEN (in press), and Miss ELSIE BODEMANN (unpublished) show that some of the gametes produced by mice heterozygous for a translocation produce non-viable embryos; non-viable, presumably, because of chromosome unbalance. Many of these embryos, particularly in the case of certain translocation stocks, die at or shortly after implantation. The nature of the abnormality causing death in these embryos has not been definitely ascertained. Others develop beyond implantation; their abnormalities, so far as we have been able to determine, are confined to the central nervous system, or to structures immediately affected in their development by

the central nervous system, and consist primarily in the failure of the neural groove to close at its anterior end. Embryos thus affected occasionally come to term, but never live more than a short time after birth.

A detailed description of the defective embryos will not be given here. It is of interest, however, to mention several abnormal individuals, not elsewhere described, whose abnormality is perhaps attributable to the translocations.

Female $F_2$390, by normal ♀L119 and semi-sterile ♂$F_1$107, exhibited a peculiar, hesitating, staggering walk, which suggested the appellation "drunken." This persisted as long as she lived. ♂$F_3$1670 and ♂$F_3$2700, half-sibs of $F_2$390 by ♀$F_2$389 and ♂$F_1$107 were still-born and showed a pronounced swelling of the top of the head, though the skin was unbroken. Fifteen progeny of $F_2$390 were normal (though two were still-born), as were the twelve sibs of ♂$F_3$1670 and ♂$F_3$2700, and fifty less closely related descendants of ♂$F_1$107.

Female F_T3914 was still-born and showed a swelling of the head similar to that described above. She was derived from test female $F_1$267, a female who was not definitely semi-sterile (average size of F_2 and F_3 litters was 7).

Unfortunately the relation of brain abnormalities to translocations had not been discovered at the time the above individuals appeared, and as a result their brains were not saved for future study. It seems not unlikely, however, that they had a mild form of brain abnormality resulting from chromosome unbalance.

It has already been noted that DOBROVOLSKAÏA-ZAVADSKAÏA (1928) found an F_1 male from treated parents the top of whose cranium was unhardened. In view of our results, this case is plausibly explained as the result of an X-ray induced deficiency.

EVIDENCE FOR THE NON-PRODUCTION OF RECESSIVE LETHAL MUTATION

The method used to test for the production or non-production of recessive lethal mutations has been described in a previous section. A lethal is indicated if mice homozygous for one of the marker genes fail to appear in the F_3 litters produced by the backcross of F_2 mice heterozygous for the marker to their F_1 parent. In no case where the tests were sufficiently extensive to be significant did the homozygous F_3 mice fail to appear. The figures on completed tests are given in the last two columns of table 5. It will be seen that 41 F_1 experimental females and 51 F_1 experimental males were tested for the absence of a lethal on one or more of the marked chromosomes. The total number of marked chromosomes tested for the absence of a lethal was 209 in the test group, 166 in the control.

TABLE 5

Summary of F_1, F_2, and F_3 mice.

	NUMBER OF F_1 PRODUCING ONE OR MORE F_2 MICE	TOTAL NUMBER OF OF F_2 LITTERS	NUMBER OF F_2 MICE					NUMBER OF F_1 PRODUCING ONE OR MORE F_2 MICE	NUMBER OF F_2 PRODUCING ONE OR MORE F_3 MICE
			LIVE-BORN		STILL-BORN				
			♀	♂	♀	♂	?		
Test	59♂♂	113	273	315	12	10	9	53♂♂	128♀♀
Test	55♀♀	77	212	241	10	3	2	46♀♀	50♂♂
Control	46♂♂	60	233	209	9	3	3	34♂♂	77♀♀
Control	57♀♀	69	237	258	9	9	4	49♀♀	49♂♂

	TOTAL NUMBER OF F_2 LITTERS	NUMBER OF F_2 MICE					MEAN SIZE OF F_2 PLUS F_3 LITTERS	ESTIMATED NUMBER OF SEMI-STERILE F_1 MICE	ESTIMATED PERCENT OF SEMI-STERILE F_1 MICE
		LIVE-BORN		STILL-BORN					
		♀	♂	♀	♂	?			
Test	211	727	722	25	16	7	6.53±.18	21♂♂	35.6
Test	113	293	319	23	15	9	5.93±.23	17♀♀	30.9
Control	115	493	502	10	10	12	8.48±.18	0♂♂	0.0
Control	105	332	342	7	4	9	6.96±.21	0??♀♀	0.0?

	NUMBER OF F_1 PRODUCING ONE OR MORE OF THE AUTOPSIED F_2 MICE	NUMBER OF F_2 PRODUCING ONE OR MORE OF THE AUTOSIPED F_2 MICE	MEAN NUMBER OF F_2 MATED TO EACH F_1	NUMBER OF AUTOPSIED F_2 MICE	MEAN NUMBER OF AUTOPSIED F_2 MICE PER F_2
Test	50♂♂	121♀♀	2.41	615	5.08
Test	41♀♀	42♂♂	1.02	254	6.05
Control	32♂♂	73♀♀	2.28	384	5.26
Control	49♀♀	49♂♂	1.00	356	7.47

	NUMBER OF F_1 WITH ONE OR MORE CHROMOSOMES TESTED FOR ABSENCE OF LETHAL	NUMBER OF CHROMOSOMES TESTED FOR ABSENCE OF LETHAL
Test	51♂♂	136
Test	41♀♀	73
Control	34♂♂	81
Control	45♀♀	85

The greatest presumption of the presence of a lethal exists in the case of control ♀ $F_1$48. A mating between this female and a son who had inherited the marker gene *p* failed to produce any *pp* offspring in a total of 13 young. The odds against this occurring by chance alone are 41 to 1. However, in view of the large number of F_1 animals being tested, such an occurrence is better explained as due to chance than as due to the presence of a lethal. In two other cases in the control and in ten cases in the treated group the recessive marker genes failed to appear, but in all these cases too few F_3 mice were raised for the results to be significant. Nine of the

ten incompletely tested mice in the treated group were semi-sterile. The small litters which they produced account for the non-completion of the tests in these nine cases.

The 209 treated chromosomes tested for the absence of a lethal are equivalent to the full chromosome complement of ten and one-half mouse spermatozoa (haploid number = 20). This number of treated sperm would yield, on the average, at least three translocations. It appears likely, therefore, that X-ray treatment of mature sperm in mice produces recessive lethals with a lower frequency, perhaps a very much lower frequency, than it produces translocations, though the possibility that some recessive lethals may have been induced on the marked chromosomes, but at loci so loosely linked with the marker loci as to escape detection, lends an element of uncertainty to this conclusion.

Whether or not translocations in mice sometimes behave as recessive lethals when homozygous, as is frequently the case in Drosophila, can only be determined by future investigations.

THE PRODUCTION OF VISIBLE MUTATIONS

Only one visible mutation, an irregular dominant causing a narrowing and constriction of the spleen and a considerable reduction in viability, was found among descendants of X-rayed animals. In affected individuals, the spleen was more or less narrowed, and showed changes in shape ranging from a slight constriction to a separation into two parts, usually unequal, giving it the shape of an exclamation point. However, some individuals, shown by progeny tests to be affected, had perfectly normal spleens. The changes in the spleen were usually visible at birth through the skin, but thereafter could be detected only by autopsy. The size of the individual as a whole was somewhat reduced, not only at birth but also in maturity. Vigor was markedly reduced, most affected individuals being very difficult to raise, and if raised, showing somewhat subnormal fertility.

Owing to the poor viability of the affected individuals, and the difficulty of detecting affected individuals except by autopsy or breeding tests, the stock was finally lost, but not until considerable data had been gathered on the inheritance of the trait. A partial pedigree of the descendants of ♂$F_1$182, the original affected male, is given in figure 3. Several lines of descent from this male have been omitted because the phenotype of some of the individuals concerned was not adequately determined. Determination at birth was often difficult, and frequently individuals suspected of being affected would die and be eaten before an autopsy was possible. The phenotype of individuals shown in the pedigree, however, is believed to have been accurately determined, unless otherwise indicated

by a question mark. The data permit of only one interpretation, namely, that the character is inherited as an irregular dominant. Several matings between affected individuals were made, but if the homozygote survived it was not detectably different from other affected individuals. It is impossible to say whether the condition was due to a mutation in the strict sense, or to a deletion as is the case with "notch" in Drosophila.

The rate of production of dominant internally visible mutations was 1 per 91 (50+41; figures from table 5, column headed "Number of F_1 producing one or more of the autopsied F_3 mice") treated spermatozoa, or approximately 1 per 1820 treated chromosomes. This is obviously very much lower than the rate of production of translocations.

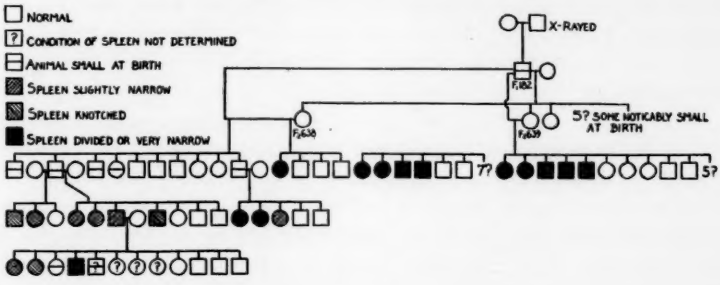

FIGURE 3.—Pedigree chart showing inheritance of X-ray-induced mutation causing alterations in the shape of the spleen, reduction in vigor and reduction in the size of the animal as a whole. Inheritance as an irregular dominant is indicated.

No other visible mutations were found. Calculation indicates that, with the system of matings used, at least one half, on the average, of the treated genes borne by F_1 individuals should segregate in the homozygous condition in the F_3. Hence at least 990 treated chromosomes were tested for recessive visible mutations detectable at birth, and 910 treated chromosomes for recessive visibles detectable only by autopsy. Since no such mutations were found, we must conclude that X-ray treatment of mouse spermatozoa, if it produces them at all, at least produces them with a much lower frequency than it produces translocations.

ABNORMALITIES PROBABLY NOT ATTRIBUTABLE TO THE X-RAY TREATMENT

A number of abnormalities were found, some of them occurring more than once, which probably were not due to the X-ray treatment.

The commonest consisted of the reduction or absence of the thirteenth rib on one or both sides. This occurred in approximately 46 percent of the short-eared dilute (ds_e/ds_e) mice of the F_3 generation, both tests and

controls, but in less than 1 percent of the non-short-eared non-dilute F_3 mice, and is therefore attributable in most cases to the short-ear or to the dilute factor, or to a factor closely linked with them.

In about 20 percent of the short-eared dilute F_3 mice, the muscular wall of the diaphragm was imperfectly formed, having a slit down the middle through which a small piece of the liver protruded into the pleural cavity. This hernia of the diaphragm occurred only in mice of the short-eared dilute phenotype, and is attributable, therefore, to the action of one or both of these genes, or of a gene closely linked with them. It is noteworthy that the gene for short-ear has already been shown (SNELL 1931) to cause alterations in the shape of the skull and a muscular waviness of the tail in addition to its primary effect on the size of the ears.

In eight F_3 mice, four of them from the control, the portal vein passed ventral to the duodenum instead of dorsal to it. In two of the cases it was ventral also to the transverse colon.

Test ♀ $F_2$724 (still-born) had a reduced upper jaw, and the lower jaw was reduced or lacking. Test ♀ $F_3$2543 (still-born) had a reduced lower jaw. Control ♀ $F_3$1689 (still-born) had almost no lower jaw. In the case of all of these females, two of the three grandparents were from the *L*-stock. Pure *L*-stock individuals occasionally are born with this same defect (agnathia). It is probably the same as the "lethal head and jaw abnormality" found by LITTLE and BAGG (1924) in both test and control lines of their X-ray experiment. The inheritance of what is probably the same trait in guinea pigs has been analysed by WRIGHT (1934).

Test ♀ $F_3$1977 had fourteen ribs on both sides. Six sibs were normal.

Test ♂ $F_3$2339 had large paired pockets, full of food, lying under the skin of the throat and opening by narrow passages into the mouth on each side of the lower jaw. Twenty-one sibs were normal.

In test ♀ $F_3$2539, the left uterus in the region of the kidney, instead of being attached to the dorsal body wall, was attached to the ventral face of the kidney itself. Seventeen sibs were normal.

Test ♀ $F_2$739 was still-born and had greatly reduced eyelids. Apparently death had occurred some little time before parturition. Seven sibs were normal. The mother, $F_1$289, was semi-sterile.

Test ♂ F_T1192 had an abnormal tail which kinked sharply up over his back due to malformation of the vertebrae. Several sibs and half sibs and numerous progeny were normal.

In test ♀ $F_3$756, the left digastricus muscle lay ventral instead of dorsal to the submaxilary gland. Sixteen sibs were normal.

Test ♂ $F_2$473 was decidedly undersize from 10 days of age until he was accidentally killed at 5 1/2 months. The shortness of the nose and shape of the head gave an appearance similar to that found in dwarf mice, though

the size was considerably larger than that of the true dwarf. The mother, $F_1$109, was semi-sterile. The two sibs that were raised were normal. One son showed similar characteristics at 2 weeks of age, but failed to survive beyond 3 weeks. Four more offspring were normal at 2 weeks when they were killed; a number were normal at birth but failed to survive.

Control ♀ F_T3512 lacked a right front leg. When examined at birth, there were a few bruises on the right side and bottom of the body, but the skin was unbroken, showing that the leg had not been eaten by the mother or lost in any similar accidental fashion. On autopsy at 28 days, it was found that the right clavicle and scapula and the upper end of the humerus were present. Thirty sibs were normal.

Control ♂ F_T1658 was still-born. Its tail consisted merely of a slender thread about one-third normal length. Examination under a dissecting binocular showed that, although the head had been eaten, no damage had been done in the region of the tail. Eight sibs, ten half-sibs, and fifteen F_3 individuals derived from the same F_1 male were all normal.

In control ♀ $F_3$367, the anterior third of the left kidney was reduced in size and showed a finer and more transparent structure than the normal part of the same kidney. The line of demarcation between the two parts was sharp. The abnormality, if it had a genetic basis at all, was perhaps the result of a somatic mutation. Four sibs were normal.

Seven of the above cases in which the abnormality appeared in one individual only were in the test group, three in the control. The difference may well have been due to chance alone; if not, slight changes in chromosome constitution of some of the individuals in the test group is the most likely explanation.

ACKNOWLEDGMENTS

The investigation reported in this paper was conceived under the stimulus of the discovery by Prof. H. J. MULLER that X-rays cause an enormous increase in the mutation rate of *Drosophila melanogaster*. Communications between the writer and Prof. MULLER revealed that almost identical plans—essentially those outlined in an above section of the paper—for an X-ray experiment with mice, had been prepared independently by each of us. Prof. MULLER, with the aid of several of his students, had developed an animal colony at the UNIVERSITY OF TEXAS for the purpose of executing this experiment. I am indebted to him for his kindness in putting this colony at my disposal. I am also indebted to him for valuable suggestions made during the course of the experiment.

Professors PATTERSON and PAINTER of the UNIVERSITY OF TEXAS cooperated in a variety of ways to aid in the conduct of the work. I wish to thank them for their assistance. Thanks are also due to the members of

the staff of the ROSCOE B. JACKSON MEMORIAL LABORATORY, to Dr. GREGORY PINCUS, and to Prof. W. H. GATES for their kindness in furnishing the stocks of mice which were used in the experiment. Miss ELSIE BODEMANN of the UNIVERSITY OF TEXAS has carried out most of the embryological studies to which reference is made in this paper. I am indebted to her for permission to refer to some of her results in advance of their publication elsewhere.

SUMMARY

1. X-rayed male mice from an inbred stock carrying five recessive genes were mated before the onset of X-ray sterility to untreated females carrying the dominant alleles of most or all of these genes. Three generations of progeny were raised, the matings being so planned as to give the greatest possible chance that any induced visible or lethal mutations would be detected. A control was provided by three generations of mice, similarly mated, and derived from the original parents of the treated group before the application of the X-rays. This paper describes results obtained in the second and third generations.

2. All individuals in the third generation, in which recessive visible mutations should appear, were autopsied with a view to the detection of mutations affecting only the internal organs.

3. Approximately 33 percent of the immediate progeny of the X-rayed males consistently produced litters of sub-normal size. This tendency to produce small litters is transmitted to later generations, and is the result of the death *in utero* of a certain proportion of the embryos. It has been designated "semi-sterility."

4. Evidence is presented showing that semi-sterility is the result of translocations carried in the heterozygous condition. The segregation of the translocations produces zygotes with chromosome unbalance which develop abnormally, and almost always die before term. The primary effect is on the central nervous system.

5. One female with a nervous disorder, and three still-born young with enlarged crania, all in the X-rayed group, were perhaps cases of relatively slight chromosome unbalance.

6. One translocation was linked with the marker gene "brown" (*b*).

7. No evidence indicating the occurrence of lethal mutations was obtained. In the test group, 209 chromosomes, in the control group 166 chromosomes, were tested and proved not to carry lethals closely linked with the marker genes.

8. One visible mutation, a variable dominant causing a reduction in width and a change in shape of the spleen, a considerable reduction in vigor, and frequently a reduction in size of the animal as a whole, was

found in the X-rayed group as a result of the autopsy. It was not determined whether the homozygote is viable. A deficiency, rather than a mutation in the strict sense, may be responsible.

9. A number of other abnormalities were found which are not attributable to the X-ray treatment.

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HETEROMORPHIC A CHROMOSOMES OF THE TOMATO DIFFERING IN SATELLITE SIZE*[1]

M. M. LESLEY and J. W. LESLEY
University of California Citrus Experiment Station, Riverside, California

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In cultivated tomato plants which were largely but not exclusively derived from triploids, chromosomes of two distinct sizes were found associated with the nucleolus in the pollen mother cells. Since a trivalent was found on the nucleolus in the simple trisomic type, triplo-A only, evidently it is the A or first chromosome which is dimorphic. At somatic metaphase this chromosome has a distinct satellite. Satellites of two sizes were found on the nucleolus in early prophase of somatic mitosis, and the difference in the length of the satellites corresponded to the difference in the length of the respective A chromosomes in the pollen mother cells. It is the length of the satellite which differs in long and short A chromosomes, the remainder of the chromosome appearing the same. Diploid plants have either a pair of long A chromosomes ("long long"), a pair of short ("short short"), or a long and a short A chromosome ("long short"). The remaining eleven pairs of chromosomes show no corresponding size difference or changed association in long A long A, short A short A, or long A short A plants.

OCCURRENCE AND TRANSMISSION OF THE TWO CHROMOSOME TYPES

Lycopersicum pimpinellifolium Mill., four races resembling *L. Humboldtii* Dun., from Mexico and Guatemala, F_1 hybrids between these and *L. pimpinellifolium*, and three races resembling *L. cerasiforme* Dun. from Mexico and southern California were short short. Single plants of the cultivated varieties King Humbert, Yellow Peach, and Norton were also in this category. Long A chromosomes were first observed in the F_2 progeny of a simple trisomic, triplo-H F_1 plant from a triploid hybrid×diploid. In F_2 the four diploid and three triplo-H plants examined were all long long, and in F_3 five of the progeny of one triplo H plant were also long long. A triploid of different origin had three long A chromosomes. F_1 hybrids between short A and long A races were long short. In F_1 from a triplo-A plant with three short A chromosomes and a long long diploid, three of the diploid plants were long short, and two triplo-A plants were short

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short long. At diakinesis in the latter, pairs of chromosomes were observed which were long short or short short, either a short or a long A chromosome remaining separate.

The size of the A chromosome is a constant character and is transmitted through the gametes to the progeny. As self-fertilization is the rule in tomatoes, after a few generations, if the three chromosome types are equally viable, long short plants would be eliminated. Long A chromosomes are not peculiar to plants derived from triploids or their derivatives in our cultures. A plant from seed sent to us by Dr. J. W. MACARTHUR of the UNIVERSITY OF TORONTO was long short. F_1 hybrids between two races sent by him and our long A races were long long so that at least one long A chromosome was present in his races. Another long long came from seed collected in Peru by Dr. S. V. JUZEPCZUK, of the Botanical Garden, Leningrad.

The progeny from selfing a long short triplo-B plant consisted of 45 diploid and 42 triplo-B plants. The A chromosomes of 47 plants taken at random were studied. They consisted of 16 long long, 18 long short, and 13 short short plants. This suggests that a long short plant forms long A and short A gametes in nearly equal proportions but that the three combinations resulting may not be equally viable. The deviation from a 1:2:1 ratio is not significant, as $X^2 = 3.0$ and $P = .23$. No characteristic phenotypic difference was found between long long, long short, and short short plants in this population or in various other populations. It is possible, however, that long long, long short, and short short plants may be distinguishable in a line which is homogeneous except in satellite size.

The A (first) chromosome of the tomato has been found to contain the loci of d_1, *p*, *o*, and *s*. The triplo-B plant C297–1 was long short and was heterozygous for d_1 and for *y*, *c*, and *u*, which MACARTHUR (1934) has found to be in the third, fourth (H), and seventh chromosomes, respectively. In the whole F_2 progeny of C297-1 the ratios were approximately

TABLE 1

Dominant and recessive phenotypes in diploid and triplo-B F_2 progeny of long-A short-A Triplo-B plant C297-1 (chromosome length was determined in part of the progeny).

A CHROMOSOMES	NUMBER OF PLANTS									
	DIPLOID	TRIPLO-B	D_1	d_1	*Y*	*y*	*C*	*c*	*U*	*u*
Long long	10	6	10	6	14	2	12	4	13	3
Long short	7	11	14	4	17	1	12	6	14	4
Short short	3	10	8	5	11	2	9	4	10	3
Not determined	24	6	18	12	16	5	23	7	16	10
Total	44	33	50	27	58	10	62	21	53	20

those of disomic inheritance (table 1). In the part which was studied cytologically, no association was found between A chromosome length and the characters determined by the D_1d_1, *Yy*, *Cc*, *Uu* gene pairs or the presence of the extra B chromosome. Short A chromosomes were found in a plant containing the three alleles D_1, *P*, and *S*, and in one containing the alleles d_1, *p*, *o*, and *s*, of the A (first) chromosome. Two plants with three short A chromosomes probably had the constitution $d_1PS/D_1pS/D_1Ps$. There is no doubt, therefore, that the d_1, *p*, *o*, and *s* loci are present in the short A chromosome.

No association was observed between the length of the A chromosomes and vigor, pollen production, or fruitfulness, except in one case which is rather suggestive. In one long long F_2 population of 123 plants, 8 diploids had almost no fruit and 4 others were intermediate between the unfruitful and the normal plants. In the unfruitful plants, no irregularities were observed in mid-prophase or later in the pollen mother cells; the pollen was normal in appearance, and these plants were otherwise indistinguishable from their diploid sibs. The F_3 progenies of two fruitful diploid and of one triplo-H parent were all normal, but the F_3 diploids from the other triplo-H parent consisted of 36 fruitful, 18 unfruitful, and 7 intermediate. In these populations inheritance of *r* and *y* was disomic, and of *c*, trisomic.

CYTOLOGICAL TECHNIQUE

Smears of pollen mother cells were fixed in Nawaschin's solution and stained in gentian violet, according to a method suggested to us by Dr. S. H. EMERSON, of the CALIFORNIA INSTITUTE OF TECHNOLOGY. In a few cases safranin or acid fuchsin was used to differentiate nucleolus and chromatin. Somatic mitosis was studied in sections of root tips 8 microns thick, using the same fixatives or the 2B fixative (LA COUR 1931) and gentian violet.

SIZE OF TOMATO CHROMOSOMES

With the exception of the long A, the chromosomes of the tomato are rather similar to one another in size both in meiosis and mitosis (plate 1, figures A-F). A study of diakinesis in pollen mother cells of some of the trisomic types has shown that the H chromosome is one of the two smallest, whereas the short A, and the B, I, and J chromosomes are relatively large. The long A chromosome is decidedly longer than any other at diakinesis. We did not find that the chromosomes of *Lycopersicum pimpinellifolium* are smaller than those of *L. esculentum* at first metaphase in pollen mother cells (plate 1, figures E and F), as described by LINDSTROM and HUMPHREY (1933). AFIFY (1933) pictures the reverse condition; chromosome size differs to this extent at metaphase in different cells

of the same smear. The four much smaller pairs figured for the tomato by COOPER (1931) have never been observed.

A CHROMOSOMES IN MEIOSIS AND SOMATIC MITOSIS

Before pachytene, while the other chromosomes are in a tangled skein the A pair consists either of a very deeply stained area near the nucleolus and slender paired threads, or the whole pair may be more deeply stained and somewhat in advance of the rest. At pachytene the A pair is associated with the nucleolus by means of a deeply stained body like that found on chromosome VI of corn and called by MCCLINTOCK (1934) the nucleolar-forming body. Extending from this body in one direction are two satellites which are usually, but not always, unpaired, broader, and more densely stained than the rest of the chromosome (plate 2, figures A-I). In the other direction from the nucleolus-forming body, which may be divided at an early stage, lies the main portion of the chromosome pair, which ends in a paler whip-like region. Separation of the pairs begins at the nucleolus-forming body and proceeds toward this paler, achromatic end. At early diakinesis the A pair is usually crossed near this end, often also near the nucleolus-forming body. At late diakinesis the pair is connected at the non-satellited end by slender crossed strands ending in small knobs which are probably derived from the pale whip-like end seen at pachytene. These knobs look like minute satellites; they can be seen only in deeply stained pollen mother cells and disappear at metaphase. The chromosomes remain attached at this end until anaphase. The true satellites cannot be distinguished in the later stages of meiosis.

In somatic metaphase short A chromosomes are distinguished by the presence of a small satellite (AFIFY 1933). Four distinct regions can be recognized: the satellite, the achromatic thread, and an intermediate region partially constricted from the fourth or main portion of the chromosome (plate 1, fig. A). This partial constriction probably indicates the position of the spindle-fiber attachment. DARLINGTON (1932, fig. 4 III N) figures a tomato chromosome with a terminal satellite and also an intercalary satellite. He says: "This chromosome has led to difficulties in counting chromosomes in Solanum, see WINKLER, 1916." We have never observed an intercalary satellite on the A chromosome of the tomato. The small terminal satellites become separated from their chromosomes in the prophase of mitosis, remaining as two compact, densely staining bodies on the surface of the nucleolus (plate 2, figures L–M), while the remainder of the short A and all of the other chromosomes are faintly stained threads. The satellites remain in this position until the disappearance of the nucleolus at the onset of metaphase. In some cells they are slightly out of the

PLATE 1

The drawings were made with the aid of a Zeiss camera lucida. The magnification is ×3070.

SOMATIC METAPHASE OF THE TOMATO

FIGURE A.—The small satellites and the attachment constriction of the short A chromosomes.

FIGURE B.—The small satellites divided, the large dividing in long A short A.

FIGURES C and D.—Typical plates of long A long A.

DIAKINESIS

FIGURE E.—*Lycopersicum esculentum*. Only one of the short A chromosomes lies on the nucleolus.

FIGURE F.—*Lycopersicum pimpinellifolium*.

FIGURE G.—*Lycopersicum peruvianum* Mill., showing two pairs of chromosomes decidedly larger than any others, both connected by unusually distinct crossed strands. The long A pair lies on the nucleolus.

FIGURE H.—Typical short A short A chromosomes at diakinesis.

FIGURE I.—Typical long A long A chromosomes at diakinesis.

FIGURE J.—Typical long A short A chromosomes at diakinesis.

Figure K.—Lateral meiotic metaphase; typical appearance of the long A long A pair and other chromosomes.

FIGURE L.—(1) Typical long A long A at first meiotic metaphase. (2) Typical short A long A at first meiotic metaphase. (3) Typical short A short A at first meiotic metaphase.

FIGURE M.—Sister plates in meiotic anaphase. The long A chromosomes are at the extreme right in each plate.

INTERKINESIS OF MEIOSIS

FIGURE N.—The long A chromosome is associated with a small nucleolar vesicle in each sister nucleus, and one larger nucleolus is present in one, two in the other.

a
b
c
d
e
g
k
f
h
l
1
2
3
i
n
m
j

plate at this stage so that twenty-six apparently separate bodies can be counted; in other cells they are clearly terminal satellites (plate 1, figure A). They are decidedly smaller than any chromosome. At anaphase they are small, deeply stained spheres and lag slightly behind the rest of the chromosomes (plate 2, figure T). They often appear to be surrounded by a clear area. Possibly these small satellites may account for the fragments found by HUSKINS and CRANE (1930) in some somatic cells of tomato rogues.

In the satellite of a long A chromosome, one can sometimes recognize at pachytene the point which corresponds to the end of the satellite of a short A (plate 2, figure F). The long A chromosome is identical with the short A except for the length of the satellite, which is approximately two-thirds longer than that of short A. Some smears of pollen mother cells of long long plants, but by no means all, show many cells with two nucleoli. In some cells a small, rounded, deeply stained body lies on one side of the long A pair, a typical nucleolus on the other; in others a small nucleolus (as indicated by its staining reaction) lies on one side, a larger one on the other (plate 2, figure I); in still others the two nucleoli are of about the same size. When two nucleoli are present, the A chromosomes always lie between them (plate 2, figure H). It seems probable that the precocious separation which begins at the satellites and proceeds through the nucleolus-forming body may divide it into two parts which, if separate enough, function independently. At diakinesis the nucleolus is always single in the tomato. At early diakinesis the long A chromosomes are normally paired at the attached end but usually diverge sharply from about the region of the nucleolus-forming body so that each chromosome may be L-shaped. The long long pair is easily recognized at meiotic metaphase both by its large size and by its peculiar shape (plate 1, figures K and L1) and can be distinguished at every succeeding stage. It sometimes lags slightly. The spindle-fiber attachment is near the base of the large satellite, and so is far nearer the center of the chromosome than in short A.

At interkinesis, following the first meiotic division, two or three small nucleoli are present in each daughter nucleus (plate 1, figure N). Since the chromosomes do not go into a resting condition one can often see the long A chromosome in each sister nucleus with a small nucleolar vesicle attached to one side. All nucleoli disappear at second metaphase.

In somatic prophase of long long plants two large satellites lie on the enormous nucleolus, which occupies about 1/9 the volume of the nucleus as compared with about 1/43 in the prophases of meiosis. Slender paired threads can be seen passing away from the satellites in some cases. The threads are often invisible because, except in lightly stained cells, the

PLATE 2

The drawings were made with the aid of a Zeiss camera lucida. The magnification is ×3070.

THE A CHROMOSOME OF THE TOMATO AT PACHYTENE AND DIPLOTENE

FIGURES A–C.—Two short A chromosomes.
FIGURES D–F.—One long and one short A chromosome.
FIGURES G–I.—Two long A chromosomes.
FIGURES J and K.—*Lycopersicum peruvianum* Mill.

THE SATELLITES OF THE A CHROMOSOME ON THE NUCLEOLUS IN SOMATIC PROPHASE

FIGURES L and M.—Short A short A.
FIGURES N and O.—Long A long A.
FIGURE P.—Long A short A.

THE LAGGING SATELLITES AT OR JUST PRECEDING SOMATIC METAPHASE

FIGURE Q.—The elongation of the nucleolus before metaphase and lagging long and short satellites in long A short A.

FIGURE R.—Lagging of two large satellites, one above and one below the plate, with consequent lagging of the remainder of the A chromosome.

FIGURE S.—Both large satellites are on one side of the forming metaphase plate.

SOMATIC ANAPHASE

FIGURE T.—The ball-shaped small satellites passing to the poles in short A short A.

FIGURE U.—Late anaphase of long A short A with one round small satellite and one lagging large one passing to each pole.

FIGURES V and W.—Late anaphase of long A long A with two large satellites lagging in each figure. They are more commonly as in W.

FIGURE X.—Early anaphase in long A short A. The large satellite just dividing, the small satellites passing toward the poles with most of the chromosomes.

FIGURE Y.—The long A chromosome at a slightly earlier stage of division.

a
b
c
d
e
f
j
g
h
i
k
n
t
l
m
o
u
q
x
y
p
v
w
r
s

nucleolus is not faint enough to allow the satellites to be distinguished. These satellites are similar in length to the smaller chromosomes but are somewhat broader and darker. They lie side by side and slightly out of the plate even at metaphase (plate 1, figures C and D) and usually appear to be separate from the long A chromosomes so that the satellites might be counted as two chromosomes. At anaphase the satellites lag consistently. They often take a deeper stain than the rest of the chromosomes and appear to be surrounded by a clear area (plate 2, figures V and W).

In meiosis, the long short pair looks at all stages just as one might expect from the description of the short short and long long. Neither chromosome influences the size of the other in the sense of dominance. They pair normally from the faint slender end to the nucleolus-forming body, and the large and small satellites are usually unpaired at pachytene (plate 2, figures D–F). At first meiotic metaphase and all succeeding stages the long A and short A chromosomes are easily distinguishable (plate 1, figure L2). The members of a pair segregate regularly at the first division.

In somatic prophase a large and a small satellite are found on the nucleolus (plate 2, figure P); and immediately preceding metaphase, while the nucleolus is disappearing and thereafter, a large oblong and a small satellite can be seen slightly out of the plate (plate 2, figure Q). As in short short, the small satellites lag somewhat, but the large ones lag decidedly behind the rest of the chromosomes as in long long. The large satellite divides more slowly than the small satellite and the rest of the A chromosome (plate 2, figures X and Y).

It is not yet clear why the satellite is more distant from its chromosome in mitosis than in meiosis. The strand appears to elongate in proportion to the size of the nucleolus and may be thought of as increasing in length in proportion to the demand made upon it or as being forced away from its chromosome by the developing nucleolus. S. NAWASCHIN (1927) states that it is longer in meristematic tissue, shorter or absent at metaphase in differentiated somatic tissue and in meiosis, and longer again in pollen cells. In other words, the strand is longer in rapidly growing and dividing cells which have large nucleoli, and shorter in less active ones which have smaller nucleoli. Longitudinal sections of tomato roots show that nucleoli are progressively smaller above and below the region of rapid mitosis.

DISCUSSION

S. NAWASCHIN (1927) has described dimorphism of the nucleolar chromosomes of *Galtonia candicans* and *Muscari tenuiflorum* caused by a much slighter difference in satellite size than that in the tomato. He found only two types of plants, one with two large satellites (symmetrical races), the

other with a large and a small satellite (asymmetrical races). He believes that the form with two small satellites is non-viable, and that with two large satellites much less viable than the asymmetrical type. MEDWEDEWA (1929) studied genetically a race of *Crepis dioscoridis*, first discovered by M. NAWASCHIN (1926), that had chromosomes differing in satellite size. By selfing he obtained the ratio of 1 ++:2 +−:1−− plants, + representing the large and − the small satellite. The ++ plants formed a marked rosette of leaves which were darker green and more deeply cut, bloomed later, and were more vigorous than −− plants; the +− plants were intermediate. The evidence indicates that in Galtonia, Crepis, and Muscari, part of a satellite is missing, and that −− plants tend to be either non-viable or less vigorous than those with a larger amount of satellite material. This is in accord with the work of BURNHAM (1932), who has shown that in maize a deficiency in a part of a satellite of chromosome VI ($Y-pl$) may reduce the viability of the eggs but does not entirely prevent them from functioning or cause abortion of the microspores. EMME (1925) found many races of Hordeum which he believed were permanently dimorphic with respect to the presence or absence of a satellite. Races were found with one or two satellites, and he concludes that plants without satellites are non-viable. In Matthiola (PHILP and HUSKINS 1931) the absence of a satellite probably acts as a pollen lethal. Apparently a certain amount of satellite material is necessary to the full development and vigor of a plant. In the tomato, a slight and non-significant deficiency of long short plants was found, but all short short plants were vigorous and fruitful and only short A chromosomes were found in all the wild races studied.

The case of the tomato shows that a considerable difference in the amount of satellite material is consistent with full vigor and fertility, and indicates that short short plants contain an adequate amount of this material. In the breeding work with the tomato, no plants have been found lacking either a whole chromosome or a chromosome fragment. The occurrence of unfruitful plants in one long long population was probably due to segregation of genes since the other long long F_2 plants and some of the F_3 progenies were entirely fruitful.

Except in Crepis, no association has been found between the phenotype and the amount of satellite material present. In the tomato, eleven of the twelve expected simple trisomic types are known and three or four types containing different chromosome fragments in addition to the normal complement. The presence even of a fragment consisting of less than half a chromosome causes a difference in the phenotype. The genetic evidence indicates that four known genes are present in the short A and that the

genes at one of these loci segregate independently of chromosome length; therefore the locus of these genes is in the region common to long and short A chromosomes. It seems probable that the extra satellite material in the long A chromosome of the tomato, as in most of the cases already cited, is genetically inert and that this condition is characteristic of satellite material. It would seem probable that a portion of a chromosome must be genetically inert before it can be either reduced in size or reduplicated without affecting a plant adversely. The Y chromosome of *Drosophila melanogaster* is characterized, as are satellites, by pycnosis and contains only one known gene (STERN 1927). The XO male fly is normal in appearance but infertile (BRIDGES 1916). The Y pairs with the X for a short distance only (METZ 1926). WILSON (1925) emphasized the fact that the Y chromosome is probably degenerating and doomed to disappear in many cases. Pycnosis and failure of complete synapsis seem to be associated, and this may be due to the absence of genes both in the Y chromosome and in satellites. Both the cytological and the genetical evidence indicate that the satellite material is different from the remainder of the A chromosome in the tomato.

Weak pairing of the terminal knobs of the satellites of maize has been observed by BURNHAM (1932) in a plant heterozygous for an interchange. The presence of chains instead of the expected rings is attributed to the fact that one of the interchanged pieces is short. It seems possible that in maize precocious shortening of the satellites interferes with pairing and prevents the formation of the expected rings. In the tomato, neither chains nor rings were found.

Evidence is accumulating to show that chromosomes with satellites, like sex chromosomes, are associated with the nucleolus at both meiosis and mitosis. HEITZ (1931b), after a careful study of the chromosomes of many species of Vicia, concludes that when a chromosome has a satellite, the nucleolus originates on the strand which connects the satellite to its chromosome. He believes that every species will be shown to possess at least one pair of chromosomes with satellites since this has been shown to be true in numerous species of the genera Crepis, Thalictrum, and Vicia. DEMOL (1928) showed that the maximum number of nucleoli in polyploid hyacinths was correlated with the number of chromosomes in the monoploid group having secondary constrictions. HEITZ (1931a) has amply confirmed this, showing further that the size and position of the nucleoli in telophase corresponds to the size and position of the satellites at anaphase. This is also true of the tomato, for in the roots of long short plants in which mitosis is proceeding rapidly, in telophase two nucleoli, one about two-thirds larger than the other, were frequently found. Cells with two

nucleoli were not seen in long long or short short root cells. It is believed that in long short plants unequal lagging of satellites leads to a difference in the time at which nucleoli are formed by the two A chromosomes. Later the two nucleoli fuse, since no cells with two nucleoli occurred in the roots of long short plants not containing division figures.

McCLINTOCK (1934) has shown that in maize a mass of chromatin which functions as a nucleolus-forming body appears on each chromosome near the satellite at pachytene. In the tomato, a similar body seems to change into a nucleolus. The position of the chromatin-rich satellite, which differs markedly in behavior and appearance from the rest of the A chromosome and is limited or lacking in gene content, suggests that the satellite may be a reservoir of material for nucleolus production. The nucleolus-forming body seems to correspond to the polar granules found on the chromosomes of Phrynotettix which WENRICH (1916) suggests are enlarged chromomeres. Each gives rise to a small nucleolus and all coalesce at the bouquet stage to form a single large nucleolus. The bouquet stage is absent in many organisms, including the tomato, and possibly it occurs only in forms in which all or several chromosomes contribute to the formation of the nucleolus. HEITZ (1931b) has shown that in Vicia nucleoli can arise from non-satellited chromosomes when those with satellites are absent.

Heteromorphy, although it has been described for autosomes, is far more frequent among nucleolar chomosomes. WILSON (1925) and SCHRADER (1928) give as cytological peculiarities of sex chromosomes heteropycnosis, lagging or early segregation, association with the nucleolus, and, in some forms, differences in size and shape. Evidently nucleolar chromosomes may possess these characteristics whether they are related to sex determination or not. According to DARLINGTON (1932), chromosomes may become differentiated as a result of fusion, fragmentation, translocation, or interchange. In the tomato, no difference in fertility, viability, or pollen development is associated with the difference in satellite size, and the extra satellite material seems to be genetically inert. It is difficult to understand how the heteromorphic condition arose, especially since, as noted above, no rings or chains of chromosomes are formed in long short plants. Satellites occur only on the A chromosomes in the tomato. Therefore, it seems probable that the added satellite material came from an A chromosome.

There is some evidence to show that the difference in the A chromosomes of the tomato may be the result of early hybridization. The tomato which was first introduced into Europe was probably unlike any known wild-type, being relatively large, ribbed, and oblate. *Lycopersicum esculentum* Mill. merely represents a group of cultivated varieties and seems to be

unknown as a really wild species. The closely related *L. pimpinellifolium* is endemic in Peru and probably in other parts of South America, but all the wild-growing races of Mexico and Guatemala are believed to have escaped from cultivation. Dr. PAUL STANDLEY, of the FIELD MUSEUM, Chicago, writes (unpublished letter) that in his opinion the tomato was introduced into Mexico before the coming of the Aztecs. The plants of *L. pimpinellifolium*, *L. Humboldtii*, and *L. cerasiforme*, which we have examined all have short A chromosomes. No probable ancestor with long A chromosomes has yet been found. It is of some interest as showing that such races may have existed, that *L. peruvianum* Mill. (*Solanum peruvianum* Jacq.), a native of Peru known as *tomate cimarrón*, seeds of which were kindly sent to us by Dr. AUGUSTO WEBERBAUER, has twelve pairs of chromosomes including a nucleolar pair with large satellites much like the long A chromosomes. One of the other pairs is also large; otherwise the chromosomes look like those of *L. esculentum* (plate 1, figure G; plate 2, figures J–K). This species is morphologically very distinct from a tomato, but its pollen applied to tomato stigmas readily causes fruit setting although the seeds are non-viable.

SUMMARY

The A (first) chromosomes of the cultivated tomato are associated with the nucleolus and are of two types, long and short, differing only in satellite size.

All wild races thus far examined have short A chromosomes. Diploid F_1 hybrids from long long×short short races have a short and a long A chromosome and in F_2 short short, long short, and long long types occur in the ratio 1:2:1. The three types are phenotypically indistinguishable, indicating that the satellites are genetically inert. The nearly related *Lycopersicum peruvianum* Mill has long A chromosomes.

The satellites are pycnotic and loosely paired at pachytene but cannot be distinguished at diakinesis. The nucleolus appears to arise on the A chromosome at the junction between the satellite and the rest of the chromosome from material which at first stains like chromatin. In prophase nuclei of meristematic cells the satellite is on or very near the nucleolus and the large satellites lie slightly out of the metaphase plate and lag at anaphase. When two nucleoli occur in long short meristematic cells, one is large, the other small, indicating a correlation between size of satellite and of nucleolus.

Both cytological and genetical evidence indicate that long and short A chromosomes are homologous. Neither shows any tendency to associate with any other chromosome.

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DOES SISTER-STRAND CROSSING OVER OCCUR IN DROSOPHILA MELANOGASTER?[1]

MORTON D. SCHWEITZER AND NATHAN KALISS
Columbia University, New York, New York

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The problem of whether sister-strand crossing over does or does not occur is of some importance for general theories of crossing over. In most genetic experiments, it is not possible to decide whether sister-strand exchange has or has not occurred because the results of both alternatives are indistinguishable. But with the use of the closed-X chromosome, described by L. V. MORGAN (1933), a solution of this question seems possible.

In heterozygous condition, the closed-X chromosome gives no offspring coming from single or triple crossing over, and there is a corresponding and fairly large proportion of eggs that fail to hatch. When total crossing over is reduced by the use of an inversion-bearing chromosome opposite the closed-X, there is observed a reduction in the frequency of inviable eggs. It was inferred (loc. cit.) that in meiosis, single and triple exchanges between a closed-X and a normal X result in chromosome attachments which give these inviable eggs. Hence, in the presence of an inversion which reduces the frequency of crossing over, fewer single and triple crossings over occur to give inviable eggs. In this case, especially if the suppression of crossing over is almost complete, the chief causes of inviable eggs would be (a) the standard inviability of the stocks, and (b) sister-strand crossing over between closed-X chromosomes. With adequate precautions and controls, the standard residual inviability could be estimated, and a direct measure obtained of the frequency of sister-strand crossing over.

The writers wish to acknowledge the kindness of L. V. MORGAN in making available stocks of the closed-X line for this experiment. One of us (N.K.) is also indebted to the DYCKMAN FUND of COLUMBIA UNIVERSITY for a grant during the summer of 1934.

METHOD

All the stocks from which the parental flies were obtained had been closely inbred for several generations prior to the beginning of the experiment.

Females for the P_1 cross were derived from seven stocks. These stocks included three inversions, (1) *ClB*, (2) delta-49, and (3) scute-8, for the

[1] This work was largely done at the MARINE BIOLOGICAL LABORATORY, Woods Hole Mass., in the summer of 1934, under the auspices of COLUMBIA UNIVERSITY.

three experimental crosses, and for the four controls the stocks (4) attached-X forked, (5) vermilion, (6) yellow-2 crossveinless vermilion forked and (7) wild-type (Columbia University stock). Virginity of each P_1 female was assured by a test period of egg-laying prior to mating. Sister females from each of the seven stocks were crossed singly to three types of males—to closed-X yellow for the experimental crosses and to vermilion and to yellow-2 crossveinless vermilion forked for the control crosses. One of each of the seven types of females was mated in turn to the selfsame male, and only those F_1 cultures were used in which all the females of the set of seven were fertilized by one male. In table 1, the genotypes of the ♀ and ♂ parents are given, together with the number of F_1 females of each type, whose egg mortality was tested. The F_1 females were mated individually to wild (C.U.) males and the eggs counted and observed for viability. In all, egg counts for 25 types on a total of 215 individual females were recorded.

TABLE 1

Crosses made and number of F_1 females used for egg counts.

♀ \ ♂	*X^c y*	*v*	*y^2 cv v f*
ClB/f fu	10	7	15
	12	8	6
N^s/y Hw dl-49	6	4	7
	6	8	5
sc^8 w^a	18	4	14
f	4	6	6
v	17	4	18
y^2 cv v f	12	10	—
+	6	2	—

The eggs, whose mortality was to be tested, were deposited on strips of colored blotting paper which had been soaked in fermenting banana juice and then overlaid with a thin layer of banana agar and mounted each on a glass slide. Blotters were changed daily, and the 1×4″ vials used were kept free from condensed moisture. Counts were made of total eggs laid and of those that had failed to hatch after 2–3 days.

The progeny of one or more days' egg-laying of 55 females were sub-

sequently examined to make sure that the frequency of the various classes that occurred was that expected for the particular cross. Every P_1 culture bottle was represented at least once among these 55, although every one of the 215 females was not tested in this way. Since no errors were found as a result of this check, and because of the precautions taken in making up the original crosses, it may be assumed that each F_1 female used for the egg counts had the chromosomal constitution indicated.

RESULTS

The frequency of inviable eggs for each type of cross is given in table 2, and the frequencies are summarized in table 3. The relative inviability of

TABLE 2

Frequency of inviable eggs.

NO.	CONSTITUTION OF F_1 FEMALE	EGGS LAID	NUMBER INVIABLE	PERCENT DIED AND STANDARD ERROR
1	*ClB/X^c y*	1828	147	8.0±9.63
2	*ClB/v*	604	45	7.5±1.07
3	*ClB/y^2 cv vf*	2105	229	10.9±0.69
4	*y Hw dl-49/X^c y*	471	19	4.0±0.90
5	*y Hw dl-49/v*	1137	38	3.3±0.53
6	*y Hw dl-49/y^2 cv vf*	1374	112	8.2±0.74
7	*sc^8 w^a/X^c y*	3301	419	12.7±0.58
8	*sc^8 w^a/v*	244	31	12.7±2.13
9	*sc^8 w^a/y^2 cv vf*	1762	193	11.0±0.75
10	*f ♀ × X^c y ♂*	229	84	36.7±3.23
11	*f ♀ × v ♂*	181	44	24.3±3.10
12	*f ♀ × y^2 cv vf ♂*	306	103	33.7±2.70
13	*N^8/X^c y*	355	127	35.8±2.54
14	*N^8/v*	310	116	37.4±2.75
15	*N^8/y^2 cv vf*	308	81	26.3±2.50
16	*ffu/X^c y*	765	170	22.2±1.50
17	*ffu/v*	461	49	10.6±1.45
18	*ffu/y^2 cv vf*	393	17	4.3±1.02
19	*v/X^c y*	136	27	20.0±3.44
20	*v/v*	278	54	19.4±2.37
21	*v/y^2 cv vf*	2277	208	9.1±0.60
22	*y^{2}cv vf/X^c y*	2225	722	32.4±0.90
23	*y^{2}cv vf/v*	655	23	3.5±0.72
24	*+/X^c y*	1236	196	15.9±1.04
25	*+/v*	181	3	1.7±0.96

the closed-X chromosome as compared with that of the normal chromosome may be estimated from the crosses to attached-X forked females (*f*). Table 2 shows 36.7 percent ± 3.23 for the closed-X as comparedwith 33.7 ± 2.70 for *y² cv v f*, and 24.3 ± 3.19 for *v* (25 percent egg inviability is due in each case to *YY* eggs.) The residual inviability for X^c *y* is therefore about that of a multiple mutant stock like *y² cv v f*. The tests with females carrying *ClB* show that the male lethal effect associated with this inversion does not cause the death of *ClB* males during the egg stage.

TABLE 3

Egg mortality summarized from table 2.

CONSTITUTION OF F_1 FEMALE	CLASSES OF TABLE 2	TOTAL EGGS	PERCENT DIED AND STANDARD ERROR
f ♀×X^c ♂	10	229	36.7±3.23
f ♀×*X* ♂	11+12	487	30.2±2.06
ClB/X^c	1	1828	8.0±0.63
ClB/*X*	2+3	2709	10.1±0.58
dl-49/X^c	4	471	4.0±0.90
dl-49/*X*	5+6	2511	6.0±0.47
sc^8/X^c	7	3301	12.7±0.58
sc^8/*X*	8+9	2006	11.2±0.68
X/X^c	16+19+22+24	4361	25.6±0.66
X/*X*	17+18+20+21+23+25	4245	8.2±0.43

The frequency of inviable eggs for all three inversions was not different from that of their respective controls. For the *ClB* and *dl*-49, the closed-X inversion was slightly lower than normal/inversion; and for scute-8, slightly in excess; but in no case was the difference as great as twice the standard error.

The absence of extra inviable eggs in any given inversion stock heterozygous for closed-X as compared with the same inversion heterozygous for normal X, can only be taken as proof that sister-strand crossing over does not occur in the absence of homologous strand crossing over. But in the absence of increased egg mortality for all three inversions, which are of different sizes and include different loci, and at least one of which, *dl*-49, allows a considerable amount of normal crossing over to occur, it may be concluded that the possibility of sister-strand crossing over is excluded for Drosophila. This conclusion is consistent with that drawn from other considerations by WEINSTEIN, MULLER and WEINSTEIN, STURTEVANT, and L. V. MORGAN.

The data given here, however, do not prove the assumed direct relation between the occurrence of single and triple crossing over in closed-X heterozygotes and the frequency of inviable eggs. To some extent, the data presented may even argue against this simple relation. In table 2, Nos. 16, 19, and 24, the frequency of inviable eggs is lower than the expected occurrence of singles. In the absence of more definite information on this relation, it therefore seems inadvisable to give an extended discussion of the questions raised.

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THE ESTABLISHMENT OF THE C_3H INBRED STRAIN OF MICE FOR THE STUDY OF SPONTANEOUS CARCINOMA OF THE MAMMARY GLAND[1]

LEONELL C. STRONG

Yale University, New Haven, Conn.

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In recent years many investigators have become interested in the use of inbred strains of mice for the study of spontaneous neoplasia. This advance has been encouraging to geneticists, who have insisted, with great reason, that intrinsic or genetic factors do play a very important role in the onset of spontaneous carcinoma of the female mammary gland. Such pioneer workers as LOEB, SLYE, LITTLE, LYNCH, and others have shown that by the proper manipulation of the process of heredity one is able to control to a very large degree the incidence of cancer in a group of mice.

The coal tar derivatives and other carcinogenic agents have given to investigators a means by which many types of neoplasia may be artificially induced at will in mice and other animals. Such divergent data, as the above, would tend to produce chaos, were it not for the significant findings obtained particularly by LYNCH (1925), by KREYBERG (1934), and by the members of the YORKSHIRE COUNCIL (1930, 31, 32, and 33). These investigators, by different means (LYNCH by hybridization, KREYBERG by use of distinctly genetic pure lines, and the Yorkshire workers by selection) have definitely shown that the response of the organism to carcinogenic materials is controlled by its own intrinsic constitution. In other words, the origin of cancer by the known carcinogenic agents is the response of the individual cell to an environmental "cancer-inciting" stimulus.

More recently, the purified hydro-carbons, such as 1-2-5-6 dibenzanthracene, benz-pyrene, etc., have been introduced into cancer research. Following the lead of the geneticists, investigators have used pure strains of mice in their sterol-painting and injection experiments. Among these may be mentioned ANDERVONT (1934) and BRANCH (1935).

In view of the difficulties of analysis, especially in this case of bringing together the contributions of divergent lines of investigations, it seems desirable at this time to present the genetic background of one strain of mice.

THE C_3H STRAIN

In 1920, the author obtained two mice from the laboratory of the department of genetics, CARNEGIE INSTITUTION OF WASHINGTON, Cold

[1] The continuation of this stock has been made possible by a grant from the JOSIAH MACY, JR., FOUNDATION.

Spring Harbor, New York. One of these (#1) was a male from the Little strain of dilute browns, the other mouse (#8) was obtained from the albino mice which Dr. H. J. BAGG had brought to the laboratory from Memorial Hospital, New York City. The past history of the Little dilute brown strain has been extensively described in the literature. The albino stock of BAGG had been imported by him from Chicago several years previously. The albino stock had been continued, up to that time, by BAGG by the non-introduction of any foreign blood. These two mice, #8 and #1, were mated during the summer of 1920. Among the numerous F_1 progeny produced by the above outcross was one female (#79) which proved to be a very interesting individual. Her history shall be briefly given. She was born December 14, 1920. Her breeding record is given in chart 1.

CHART 1.

This chart presents the breeding record of mouse #79 which was first mated to her own brother #77 and then to a son #352 obtained from the above cross.

LITTER SEQUENCE	MATED TO MALE	LITTER BORN	NUMBER IN LITTER	YOUNG RAISED
1	77	2–12–21	5	2
2	77	4–20–21	7	4
3	77	6– 8–21	7	5
4	77	7–22–21	10	8
5	77	9–12–21	6	4
6	352 (own son)	12–20–21	7	7
7	352 (own son)	1–29–22	8	6
8	352 (own son)	3– 9–22	?	Litter killed by mother

On the twenty-second of October, 1921, a nodule 0.8 cm. in diameter was noticed in the left axillary region (2nd gland). A second nodule 0.5 cm. in diameter was discovered in the right axillary region on the twenty-fifth of the same month. The mouse was operated on under ether anesthesia on the twenty-ninth of October. The original tumor was a compact adenocarcinoma. Since this was the fourth tumor to be discovered in an F_1 individual, the original tumor received the symbol F_1Da. The tumor was inoculated into several offsprings of mouse #79, and into other F_1 individuals. On the sixth of January, 1922, two other nodules had appeared in mouse #79. These were in the left (F_1Dc) and the right iliac (F_1Dd) regions respectively (the 4th nipple systems). By the twenty-fourth of January, 1922, the second or F_1Db tumor had reached a centimeter and a half in diameter. This was removed by operation on that date—and subsequently recurred in the scar tissue by the fourth of March of that year. This tumor was inoculated into several F_1 individuals and into close relatives of #79.

The third tumor (F_1Dc) in the left iliac region was also removed on the twenty-fourth of January. At that time it was a centimeter in diameter. A fifth tumor, F_1De, approximately a centimeter in diameter was noticed on the nape of the neck on the fourth of March, 1922. To the left of this nodule another one (F_1Df) occurred in the right axillary region. The mouse died on the seventeenth of March at which time four surface tumors were readily palpated. These were F_1De, F_1Df, F_1Dg, and a recurrence of F_1Db. Autopsy disclosed three metastatic nodules in the lungs. All other organs were apparently normal. Attempts to continue most of the tumors by transplantation led to indifferent success. Two tumors, F_1Da and F_1Dg, were continued by transplantation for several years. The data obtained with these tumors were reported in the Journal of Cancer Research, 1929.

The conclusion derived from this study was that the tumor tissue had deviated from the definitive somatic cell that gave rise to it by a process analogous to genetic mutation. The question whether these nodules were multiple primary or secondary nodules is of extreme genetic interest. In the transplantation work it was clearly demonstrated that distinctly physiological differences were encountered in these tumors derived from the same mouse. Thus, it is true that the same mouse may give rise to genetically different types of tumors at approximately the same time.

In addition to the possibility of working out genetically the fundamental differences of physiological behavior of these tumors by the process of transplantation, it occurred to the author that mouse #79 may be of interest in the study of the origin of spontaneous carcinomata. With this end in view, the progeny obtained from mouse #79 by the method illustrated in chart 2 have been kept normally in the laboratory until they either died or developed spontaneous carcinoma. In the early years I was interested not only in the spontaneous occurrence of carcinoma, but also in the working out of the genetics of transplantation of the two implants, F_1Da and F_1Dg. Thus, the author decided to produce, if possible, a strain of mice which had genetic constitutions similar to that of mouse #79. For this reason mouse #79 was mated to her own brother and then mated to her own son, #352, obtained by this cross. Among the progeny obtained, four females were mated to a single male (a brother). After several generations of transplantation the implants F_1Da and F_1Dg were lost. The interest than centered on the occurrence of spontaneous carcinoma of the female mammary gland. Hence, the line was continued primarily by a brother-to-sister mating. Two breaks have occurred in this system of inbreeding. Each one was necessitated by the loss of animals when the colony was transferred from one laboratory to another. The first break was the mating of mouse 11113 to her near relative 11242 shortly after the move to Ann Arbor, Michigan, and the second break the mating of mouse

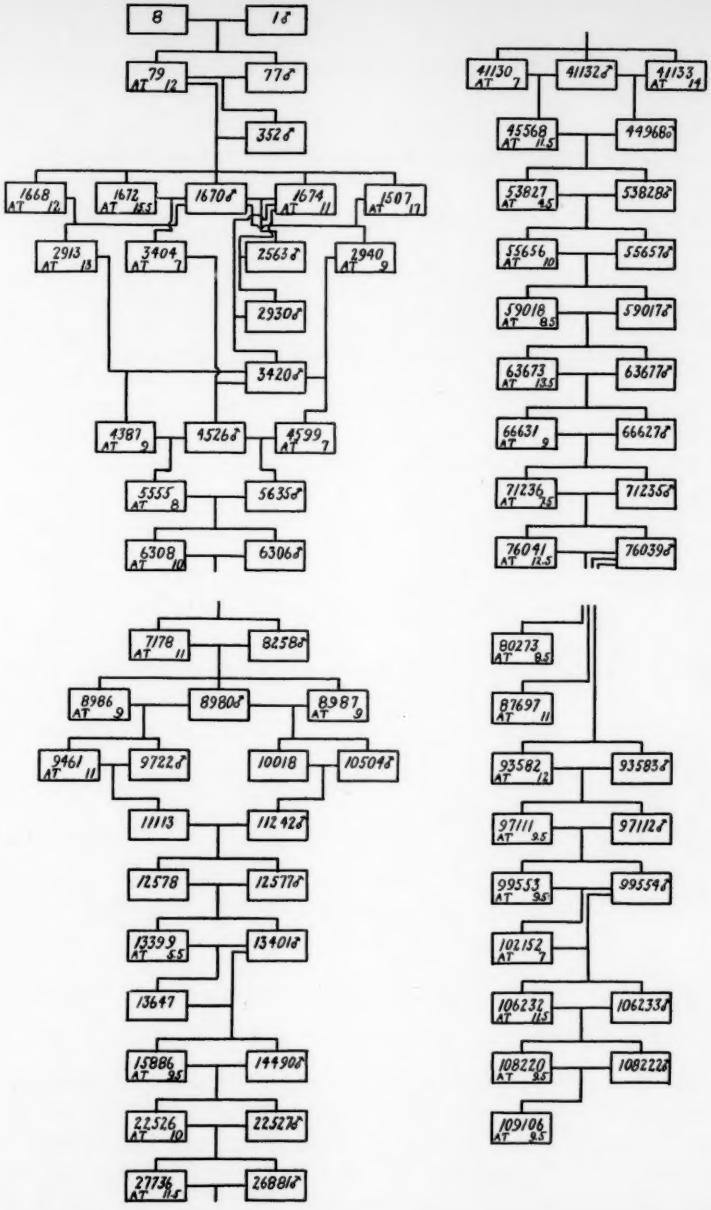

CHART 2

Males on the chart are indicated by the sign ♂. All other mice represented are females. The age at which spontaneous carcinoma of the mammary gland appeared is indicated below the serial number of the female. If the space is blank below the number of the female, the mouse died of some other cause than cancer. Only mice that are genetically related and which therefore constitute strain C_3H are included in this chart.

76039 not only to his own sister, but to the ensuing daughter and finally his own granddaughter necessitated by the move to Bar Harbor, Maine.

It will be noted that every female in direct descent for the last twenty-one generations has developed spontaneous carcinoma of the mammary gland. The several hundred descendents of mouse #79 in collateral lines, together with the numerous appearances of spontaneous carcinoma of the mammary gland in these mice, is not included in this report.

Of the forty-two female descendants of mouse #79, in this single line of descent, thirty-eight developed primary carcinoma of the mammary gland.

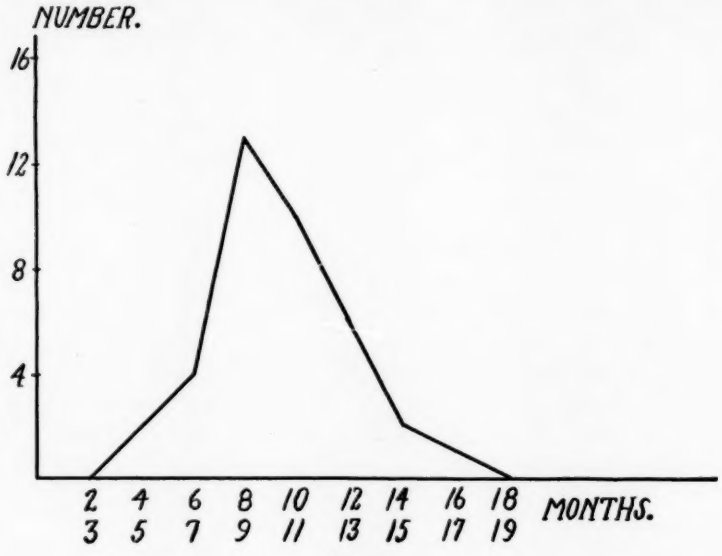

CHART 3

Age distribution of spontaneous carcinoma of the mammary gland in mice of the C_3H strain.

The age distribution of cancer is given in chart 3. The majority of the mice developed tumors between seven and ten months, with the mean between eight and nine months. This is earlier in life than data reported by SLYE (1927), MARSH (1929), and MURRAY (1934) on mammary gland carcinoma in other laboratories.

Derivation of Symbol C_3H

After a fair degree of inbreeding had been established, it seemed desirable to designate the various sub-strains thus obtained by letters of the alphabet. Consequently the descendents of mouse #5555 were designated

by the letter C. Two generations beyond, a further classification became desirable. Thus the descendents of mouse #8986 received the designation C_3H.

GENERAL DISCUSSION

The carcinogenic agents derived from coal tar produce several distinct types of neoplasia. The more frequent types are, however, epithelioma of the skin and sarcoma. Lynch has demonstrated that the response of the different organs of the body to neoplasia is apparently inherited independent of each other.

The purposes to which the inbred strains of mice, such as the above, can be utilized, are (1) the study of the incidence of spontaneous carcinoma, and (2) the study of the production of neoplasia by the recognized carcinogenic agents. The extent to which the above strain has been inbred is certainly sufficient to approach genetic homogeneity. In other words, biological variability has been reduced to a minimum.

The C_3H strain is to be considered a highly susceptible cancer family only so far as cancer of the female mammary gland is concerned. In regard to the tumors of the other organs it is still to be considered a resistant strain—since tumors other than those of the mammary gland have never been encountered in a period of fifteen years.

CONCLUSION

1. The lineal descendents of mouse #8986 constitute strain C_3H.
2. The C_3H strain, established by a system of pedigreed inbreeding over a period of fifteen years, is to be considered a pure line in regard to the occurrence of spontaneous carcinoma of the female mammary gland.

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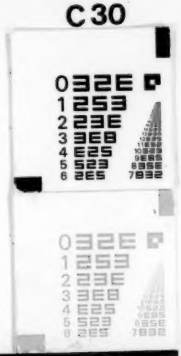
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